

Docket # PF-0513-1 DIV

11/14/00



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By: Nancy RamosPrinted: Nancy Ramos

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING A PATENT APPLICATION UNDER 37 CFR 1.53(b)**

Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

Dear Sir:

This is a request for filing a **DIVISIONAL** application under 37 CFR 1.53(b) of pending prior application Serial No. 09/071,709, filed on May 1, 1998, entitled **HUMAN PROTEASE ASSOCIATED PROTEINS**.

1. X Enclosed is a specification corresponding to the prior application, U. S. Application Serial No. 09/071,709 filed May 1, 1998, including the oath or declaration as originally signed. The specification does not contain any subject matter that would have been new matter in the prior application.
2. X With regard to the requirement of 37 CFR 1.821(e) which requires that a copy of the Sequence Listing in computer readable form (CRF) be submitted, Applicants state that the paper copy of the Sequence Listing for the instant divisional application is identical with the computer readable form filed with Serial No. 09/071,709, filed May 1, 1998, to which priority is claimed. In accordance with 37 C.F.R. § 1.821(e), please use the computer readable form filed with U.S. Application Serial No. 09/071,709 as the computer readable form for the instant divisional application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant divisional application.
3. X The inventor(s) of the invention being claimed in this application is (are): Jennifer L. Hillman; Y. Tom Tang; Preeti Lal; Neil C. Corley; Karl J. Guegler; Chandra Patterson.
4. X In accordance with 37 CFR 1.63(d) a copy of the originally signed declaration showing applicant's/applicants' signature(s) as filed on August 11, 1998 is enclosed.

5. X Amend the specification by inserting after the title: "This application is a divisional application of U.S. application serial number 09/071,709, filed May 1, 1998, all of which applications and patents are hereby incorporated herein by reference."
6. X The filing fee is calculated below:

Claims	Number Filed	Minus	Number Extra	Other Than Small Entity Rate Fee		Basic Fee
						\$710.00
Total Claims	19	-20	0	x \$18	0	\$0
Indep. Claims	3	-3	0	x \$80	0	\$0
Multiple Dependent Claim(s), if any				+ \$270		\$0

TOTAL FILING FEE \$710.00

7. ___ An extension of time in the above-named prior application has been requested and the fees therefore have been authorized in said application.
8. X Please charge Deposit Account No. **09-0108** in the amount of \$710.00 .
- The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to Deposit Account No. **09-0108**.
- A duplicate copy of this Request is enclosed.
9. X New formal drawings are enclosed.
10. X The prior application is assigned of record to Incyte Pharmaceuticals, Inc., recorded on August 11, 1998, at reel 9402/frame 0915.
11. ___ A preliminary amendment is enclosed.
12. X Also enclosed is an Information Disclosure Statement and Form PTO-1449.
13. X The power of attorney of the prior application is to:

Lucy J. Billings
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Sheela Mohan-Peterson
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Reg. No. 36,749
Reg. No. 39,132
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Reg. No. 37,071

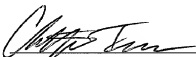
___ The associate power of attorney in the prior application is to:

- a. X A new power of attorney is attached.
- b. ___ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. X Address all future correspondence to:

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X Filed under 37 CFR 1.34(a)

Registration number if acting under 37 CFR 1.34(a) 45,167

PF-0513-1 DIV

HUMAN PROTEASE ASSOCIATED PROTEINS

This application is a divisional of USSN 09/071,709, filed May 1, 1998.

FIELD OF THE INVENTION

This invention relates to nucleic acid and amino acid sequences of human protease associated proteins and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative and immune disorders .

BACKGROUND OF THE INVENTION

Proteolytic processing is an essential component of normal cell growth, differentiation, remodeling, and homeostasis. The cleavage of peptide bonds within cells is necessary for a variety of functions, including the maturation of precursor proteins to their active form, the removal of signal sequences from targeted proteins, the degradation of incorrectly folded proteins, and the controlled turnover of peptides within the cell. Proteases participate in apoptosis, inflammation, and in tissue remodeling during embryonic development, wound healing, and normal growth. They are necessary components of bacterial, parasitic, and viral invasion and replication within a host. Mammalian proteases have been identified and categorized based on active site structure, mechanism of action, and overall three-dimensional structure. (Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York, NY, pp. 1-5.)

The serine proteases (SPs) are a large family of proteolytic enzymes that include the digestive enzymes, trypsin and chymotrypsin; components of the complement and blood-clotting cascades; and enzymes that control the degradation and turnover of macromolecules of the extracellular matrix. SPs are named because of the presence of a serine residue found in the active catalytic site which forms a triad together with an aspartate and a histidine residue. SPs have a wide range of substrate specificities and can be subdivided into subfamilies on the basis of these specificities. The main sub-families are trypases, which cleave after arginine or lysine; aspages, which cleave after aspartate; chymases, which cleave after phenylalanine or leucine; metases which cleave after methionine; and serases, which cleave after serine.

Cysteine proteases are involved in diverse cellular processes ranging from the processing of precursor proteins to intracellular degradation. Mammalian cysteine proteases include lysosomal cathepsins and cytosolic calcium activated proteases, calpains. Cysteine proteases are produced by monocytes, macrophages and other cells of the immune system which migrate to sites of inflammation and secrete various molecules necessary for the repair of damaged tissue. These cells may overproduce the same molecules and cause tissue destruction in certain disorders. For example,

in autoimmune diseases such as rheumatoid arthritis, the secretion of the cysteine protease, cathepsin C, degrades collagen, laminin, elastin and other structural proteins found in the extracellular matrix of bones.

Carboxypeptidases A and B are the principal mammalian representatives of the metallo-protease family. Both are exopeptidases of similar structure and active sites. Carboxypeptidase A, like chymotrypsin, prefers C-terminal aromatic and aliphatic side chains of hydrophobic nature, whereas carboxypeptidase B is directed toward basic arginine and lysine residues. Active site components include zinc, with its three ligands of two glutamic acid and one histidine residues.

Many other proteolytic enzymes do not fit any of the major categories discussed above because their mechanisms of action and/or active sites have not been elucidated. These include the aminopeptidases and signal peptidases. Aminopeptidases catalyze the hydrolysis of amino acid residues from the amino terminus of peptide substrates. Bovine leucine aminopeptidase is a zinc metallo-enzyme that utilizes the sulfhydryl groups from at least three reactive cysteine residues at its active site in the binding of metal ions. (Cuyper, H.T. et al. (1982) J. Biol. Chem. 257:7086-7091.)

Signal peptidases are a specialized class of proteases found in all prokaryotic and eukaryotic cell types that serve in the processing of signal peptides from certain proteins. Signal peptides are amino-terminal sequences on a protein which directs the protein from its ribosomal assembly site to a particular cellular or extracellular location. Once the protein has been exported, removal of the signal sequence by a signal peptidase and posttranslational processing, e.g., glycosylation or phosphorylation, activate the protein. Signal peptidases exist as multi-subunit complexes in both yeast and mammals.

Proteasome, an intracellular protease complex found in some bacteria and in all eukaryotic cells, plays an important role in cellular physiology. Proteasomes are responsible for the timely degradation of cellular proteins of all types and control proteins that function to activate or repress cellular processes such as transcription and cell cycle progression. (Ciechanover, A. (1994) Cell 79:13-21.) Proteasomes act on proteins which have been targeted for hydrolysis by the covalent attachment of a small protein called ubiquitin to lysine side chains of the protein. Ubiquitin-proteasome systems are implicated in the degradation of mitotic cyclic kinases, oncoproteins, tumor suppressor genes (p53), cell surface receptors associated with signal transduction, transcriptional regulators, and mutated or damaged proteins. (Ciechanover, supra.) Proteasomes are large (~ 2000 kDa), multisubunit complexes composed of a central catalytic core containing a variety of proteases and terminal subunits that serve in substrate recognition and regulation of proteasome activity.

Protease inhibitors play a major role in the regulation of the activity and effect of proteases. They have been shown to control pathogenesis in animal models of proteolytic disorders. (Murphy, G. (1991) Agents Actions Suppl 35:69-76.) In particular, low levels of the cystatins, low molecular weight inhibitors of the cysteine proteases, seem to be correlated with malignant progression of tumors. (Calkins, C. et al (1995) Biol Biochem Hoppe Seyler 376:71-80.) The balance between levels of cysteine proteases and their inhibitors is also significant in the development of disorders. Specifically, increases in cysteine protease levels, when accompanied by reductions in inhibitor activity, are correlated with increased malignant properties of tumor cells and the pathology of arthritis and immunological diseases in humans.

The Kunitz family of serine protease inhibitors are characterized by one or more "Kunitz domains" containing a series of cysteine residues that are regularly spaced over approximately 50 amino acid residues and form three intrachain disulfide bonds. Members of this family include aprotinin, tissue factor pathway inhibitor (TFPI-1 and TFPI-2), inter- α -trypsin inhibitor, and bikunin. (Marlor, C.W. et al. (1997) J. Biol. Chem. 272:12202-12208.) Members of this family are potent inhibitors (in the nanomolar range) against serine proteases such as kallikrein and plasmin. Aprotinin has clinical utility in reduction of perioperative blood loss.

The discovery of new human protease associated proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of cell proliferative and immune disorders.

SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, human protease associated proteins, referred to collectively as "HPRAP" and individually as "HPRAP-1", "HPRAP-2", "HPRAP-3", and "HPRAP-4." In one aspect, the invention provides a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

The invention further provides a substantially purified variant having at least 90% amino acid identity to the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4, or to a fragment of any of these sequences. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4. The invention also includes an isolated and purified polynucleotide variant having at least

90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4, as well as an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, a fragment of SEQ ID NO:5, a fragment of SEQ ID NO:6, a fragment of SEQ ID NO:7, and a fragment of SEQ ID NO:8. The invention further provides an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, a fragment of SEQ ID NO:5, a fragment of SEQ ID NO:6, a fragment of SEQ ID NO:7, and a fragment of SEQ ID NO:8, as well as an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, a fragment of SEQ ID NO:5, a fragment of SEQ ID NO:6, a fragment of SEQ ID NO:7, and a fragment of SEQ ID NO:8.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a

fragment of SEQ ID NO:4, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

5 The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4 in conjunction with a suitable pharmaceutical carrier.

10 The invention further includes a purified antibody which binds to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4, as well as a purified agonist and a purified antagonist to the polypeptide.

15 The invention also provides a method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

20 The invention also provides a method for treating or preventing an immune disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4.

25 The invention also provides a method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence of SEQ ID NO:2, or a fragment of SEQ ID NO:2.

30 The invention also provides a method for treating or preventing an immune disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence of SEQ ID NO:2, or a fragment of SEQ ID NO:2.

The invention also provides a method for detecting a polynucleotide encoding the

polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4 in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide sequence encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, a fragment of SEQ ID NO:1, a fragment of SEQ ID NO:2, a fragment of SEQ ID NO:3, and a fragment of SEQ ID NO:4 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide in the biological sample. In one aspect, the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, 1C, 1D, 1E and 1F show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:5) of HPRAP-1. The alignment was produced using MACDNASIS PRO software (Hitachi Software Engineering Co. Ltd., San Bruno, CA).

Figures 2A, 2B, 2C, 2D, and 2E, show the amino acid sequence (SEQ ID NO:2) and nucleic acid sequence (SEQ ID NO:6) of HPRAP-2. The alignment was produced using MACDNASIS PRO software.

Figures 3A, 3B, and 3C, show the amino acid sequence (SEQ ID NO:3) and nucleic acid sequence (SEQ ID NO:7) of HPRAP-3. The alignment was produced using MACDNASIS PRO software (Hitachi Software Engineering).

Figures 4A, 4B, 4C, 4D, and 4E, show the amino acid sequence (SEQ ID NO:4) and nucleic acid sequence (SEQ ID NO:8) of HPRAP-4. The alignment was produced using MACDNASIS PRO software (Hitachi Software Engineering).

Figures 5A, 5B, 5C, and 5D show the amino acid sequence alignments between HPRAP-1 (031381; SEQ ID NO:1) and an endooligopeptidase A related protein from Oryctolagus cuniculus (GI 2827886; SEQ ID NO:9), produced using the multisequence alignment program of LASERGENE software (DNASTAR Inc, Madison WI).

Figures 6A and 6B show the amino acid sequence alignments between HPRAP-2 (1319265; SEQ ID NO:2) and the kunitz type protease inhibitor, bikunin, from human (GI 2065529; SEQ ID NO:10), produced using the multisequence alignment program of LASERGENE software (DNASTAR).

Figures 7A and 7B show the amino acid sequence alignments between HPRAP-3 (2057812; SEQ ID NO:3) and the human proteasome subunit, p27 (GI 2055256; SEQ ID NO:11), produced using the multisequence alignment program of LASERGENE software (DNASTAR).

Figures 8A, 8B, and 8C show the amino acid sequence alignments between HPRAP-4 (529706; SEQ ID NO:4) and a vacuolar aminopeptidase-related protein from Caenorhabditis elegans (GI 529706; SEQ ID NO:12), produced using the multisequence alignment program of LASERGENE software (DNASTAR).

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"HPRAP," as used herein, refers to the amino acid sequences of substantially purified HPRAP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist," as used herein, refers to a molecule which, when bound to HPRAP, increases or prolongs the duration of the effect of HPRAP. Agonists may include proteins, nucleic

acids, carbohydrates, or any other molecules which bind to and modulate the effect of HPRAP.

An "allelic variant," as this term is used herein, is an alternative form of the gene encoding HPRAP. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered.

- 5 Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

- 10 "Altered" nucleic acid sequences encoding HPRAP, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide encoding a polypeptide the same as HPRAP or a polypeptide with at least one functional characteristic of HPRAP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HPRAP, and improper or unexpected hybridization to allelic variants, with a locus other than the
15 normal chromosomal locus for the polynucleotide sequence encoding HPRAP. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HPRAP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or
20 immunological activity of HPRAP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

- 25 The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of HPRAP which are preferably about 5 to about 15 amino acids in length, most preferably 14 amino acids, and which retain some biological activity or immunological
30 activity of HPRAP. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

5 The term "antagonist," as it is used herein, refers to a molecule which, when bound to HPRAP, decreases the amount or the duration of the effect of the biological or immunological activity of HPRAP. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of HPRAP.

10 As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind HPRAP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired.

15 Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein

20 is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence.

25 Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

30 As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic HPRAP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind

with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity
 5 between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and
 10 in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising polynucleotide
 15 sequences encoding HPRAP or fragments of HPRAP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts, e.g., NaCl, detergents, e.g., sodium dodecyl sulfate (SDS), and other components, e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.

"Consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended in the 5' and/or the 3' direction using an XL-PCR kit (Applied Biosystems, Foster City CA) and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison, WI). Some sequences
 20 have been both extended and assembled to produce the consensus sequence.

As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding HPRAP, by Northern analysis is indicative of the presence of nucleic acids encoding HPRAP in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding
 25 HPRAP.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative," as used herein, refers to the chemical modification of a polypeptide

sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "similarity," as used herein, refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MegAlign program, a component of LASERGENE software (DNASTAR). The MegAlign program can create alignments between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) *Gene* 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic

acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) *Methods Enzymol.* 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

5 “Human artificial chromosomes” (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) *Nat Genet.* 15:345-355.)

10 The term “humanized antibody,” as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

 “Hybridization,” as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

15 As used herein, the term “hybridization complex” refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C₀t or R₀t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

20 The words “insertion” or “addition,” as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

25 “Immune response” can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

 The term “microarray,” as used herein, refers to an arrangement of distinct polynucleotides arrayed on a substrate, e.g., paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

30 The terms “element” or “array element” as used herein in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

 The term “modulate,” as it appears herein, refers to a change in the activity of HPRAP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics,

or any other biological, functional, or immunological properties of HPRAP.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" or "operably linked," as used herein, refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) *Anticancer Drug Des.* 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding HPRAP, or fragments thereof, or HPRAP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a solid support; a tissue; a tissue print; etc.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is

dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent (e.g., formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least

about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of HPRAP, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

THE INVENTION

The invention is based on the discovery of new human protease associated proteins (HPRAP), the polynucleotides encoding HPRAP, and the use of these compositions for the diagnosis,

treatment, or prevention of cell proliferative and immune disorders.

Nucleic acids encoding the HPRAP-1 of the present invention were first identified in Incyte Clone 031381 from the promonocyte cell line cDNA library (THP1NOB01) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:5, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 031381 (THP1NOB01), 3618224 (EPIPNOT01), 991115 (COLNNOT11), 1223519 (COLNTUT02), 1723492 (BLADNOT06), 599353 (BRSTNOT02), 2132359 (OVARNOT03), 853456 (NGANNT01), and 673630 (CRBLNOT01).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figures 1A, 1B, 1C, 1D, 1E, and 1F. HPRAP-1 is 460 amino acids in length and has a potential N-glycosylation site at residue N299, and potential phosphorylation sites for cAMP- and cGMP-dependent protein kinase at T132, for casein kinase II at S11, S29, S95, S135, S155, and S310, for protein kinase C at S11, S23, S90, T191, S231, S247, S282, S331, S344, and S404, and for tyrosine kinase at Y87. As shown in Figures 5A, 5B, 5C, and 5D, HPRAP-1 has chemical and structural similarity with an endooligopeptidase A related protein from *O. cuniculus* (GI 2827886; SEQ ID NO:9). In particular, HPRAP-1 and the endooligopeptidase A related protein share 89% identity, including the potential N-glycosylation site and most of the potential phosphorylation sites found in HPRAP-1. The fragment of SEQ ID NO:5 from about nucleotide 1152 to about nucleotide 1205 is useful, for example, as a hybridization probe. Northern analysis shows the expression of this sequence in various libraries, at least 48% of which are immortalized or cancerous and at least 36% of which involve immune response. Of particular note is the expression of HPRAP-1 in cardiovascular and gastrointestinal tissues.

Nucleic acids encoding the HPRAP-2 of the present invention were first identified in Incyte Clone 1319265 from the bladder tissue cDNA library (BLADNOT04) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:6, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 1319265 (BLADNOT04), 1522685 (BLADTUT04), 872599 and 875890 (LUNGAST01), 1427703 (SINTBST01), 1224264 (COLNTUT02), and 1493438 (PROSNON01).

In another embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2, as shown in Figures 2A, 2B, 2C, 2D, and 2E. HPRAP-2 is 349 amino acids in length and has two potential N-glycosylation sites at residues N53 and N342, and potential phosphorylation sites for casein kinase II at T43, S169, S193, S264, S331, and T332, and for protein

kinase C at S60, S71, S205, S259, S264, and T344. HPRAP-2 contains a potential signal peptide sequence between residues M1 and P48 and a potential transmembrane domain between residues M285 and C305. HPRAP-2 also contains two sequences related to the Kunitz family domains between residues C94 and C119, and residues C235 and C260. As shown in Figures 6A and 6B,

5 HPRAP-2 has chemical and structural similarity with the kunitz type protease inhibitor, bikunin, from human (GI 2065529; SEQ ID NO:10). In particular, HPRAP-2 and human bikunin share 25% identity, including 12 cysteine residues located in the two kunitz domains in both proteins. The fragment of SEQ ID NO:6 from about nucleotide 500 to about nucleotide 563 is useful, for example, as a hybridization probe. Northern analysis shows the expression of this sequence in various
10 libraries, at least 60% of which are immortalized or cancerous and at least 41% of which involve immune response. Of particular note is the expression of HPRAP-2 in reproductive and gastrointestinal tissues.

Nucleic acids encoding the HPRAP-3 of the present invention were first identified in Incyte Clone 2057812 from the bronchial epithelium cDNA library (BEPINOT01) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:7, was
15 derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2057812 (BEPINOT01), 2496092 (ADRETUT05), and 026889 (SPLNFET01).

In another embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:3, as shown in Figures 3A, 3B, and 3C. HPRAP-3 is 239 amino acids in
20 length and has a potential N-glycosylation site at residue N204, and potential phosphorylation sites for casein kinase II at S2, T19, and S65, and for protein kinase C at S46, T74, and S121. As shown in Figures 7A and 7B, HPRAP-3 has chemical and structural similarity with the human proteasome subunit, p27 (GI 2055256; SEQ ID NO:11). In particular, HPRAP-3 and p27 share 87% identity. The fragment of SEQ ID NO:7 from about nucleotide 551 to about nucleotide 604 is useful, for
25 example, as a hybridization probe. Northern analysis shows the expression of this sequence in various libraries, at least 51% of which are immortalized or cancerous and at least 36% of which involve immune response. Of particular note is the expression of HPRAP-3 in cardiovascular, hematopoietic/immune, nervous, and reproductive tissues.

Nucleic acids encoding the HPRAP-4 of the present invention were first identified in Incyte
30 Clone 2058485 from the ovarian tissue cDNA library (OVARNOT03) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:8, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2058485 (OVARNOT03), 3115916 (LUNGUT13), 556715 (MPHGLPT02), 2023129 (CONNNOT01),

1513114 (PANCTUT01), 2023129 (CONNNOT01), and 1964262 (BRSTNOT04).

In another embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:4, as shown in Figures 4A, 4B, 4C, 4D, and 4E. HPRAP-4 is 471 amino acids in length and has a potential N-glycosylation site at residue N65, and potential phosphorylation sites for cAMP- and cGMP-dependent protein kinase at S102 and S320, for casein kinase II at T124, S227, T292, T327, and S437, and for protein kinase C at T49, S102, S143, S227, and S437. As shown in Figures 8A, 8B, and 8C, HPRAP-4 shares chemical and structural similarity with a vacuolar aminopeptidase-related protein from *C. elegans* (GI 529706; SEQ ID NO:12). In particular, HPRAP-4 and the vacuolar aminopeptidase-related protein share 46% identity. Fragments of SEQ ID NO:8 from about nucleotide 254 to about nucleotide 319, and from about nucleotide 1025 to about nucleotide 1097 are useful, for example, as hybridization probes. Northern analysis shows the expression of this sequence in various libraries, at least 50% of which are immortalized or cancerous and at least 32% of which involve immune response. Of particular note is the expression of HPRAP-4 in reproductive and gastrointestinal tissues.

The invention also encompasses HPRAP variants. A preferred HPRAP variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the HPRAP amino acid sequence, and which contains at least one functional or structural characteristic of HPRAP.

The invention also encompasses polynucleotides which encode HPRAP. In a particular embodiment, the invention encompasses a polynucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, which encodes an HPRAP.

The invention also encompasses a variant of a polynucleotide sequence encoding HPRAP. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding HPRAP. A particular aspect of the invention encompasses a variant of SEQ ID NO:2 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of HPRAP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HPRAP, some bearing minimal

similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HPRAP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HPRAP and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HPRAP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HPRAP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HPRAP and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HPRAP and HPRAP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HPRAP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, a fragment of SEQ ID NO:5, a fragment of SEQ ID NO:6, a fragment of SEQ ID NO:7, and a fragment of SEQ ID NO:8 under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.)

Methods for DNA sequencing are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE, Taq DNA polymerase and thermostable T7 DNA polymerase (Amersham Pharmacia Biotech (APB), Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Rockville MD). Preferably, the process is automated with machines such

as the MICROLAB 2200 system (Hamilton, Reno NV) and the DNA ENGINE thermal cycler (MJ Research, Watertown MA) and the ABI PRISM 3700, 373, and 377 DNA sequencing systems (Applied Biosystems).

The nucleic acid sequences encoding HPRAP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto, CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO primer analysis software (Molecular Biology Insights, Inc. (MBI), Cascade CO) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the

emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Applied Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HPRAP may be cloned in recombinant DNA molecules that direct expression of HPRAP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HPRAP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HPRAP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HPRAP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) *Nucleic Acids Symp. Ser. (7):215-223*, and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser. (7):225-232*.) Alternatively, HPRAP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Applied Biosystems). Additionally, the amino acid sequence of HPRAP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman and Co., New York, NY.)

In order to express a biologically active HPRAP, the nucleotide sequences encoding HPRAP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which

contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and polynucleotide sequences encoding HPRAP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HPRAP. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding HPRAP and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HPRAP and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding HPRAP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HPRAP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HPRAP can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT plasmid (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding HPRAP into the vector's

multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HPRAP are needed, e.g. for the production of antibodies, vectors which direct high level expression of HPRAP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HPRAP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, supra; and Grant et al. (1987) Methods Enzymol. 153:516-54; Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HPRAP. Transcription of sequences encoding HPRAP may be driven by viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987) EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding HPRAP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HPRAP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino

polymers, or vesicles) for therapeutic purposes.

For long term production of recombinant proteins in mammalian systems, stable expression of HPRAP in cell lines is preferred. For example, sequences encoding HPRAP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*⁻ and *aprt*⁻ cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14; and Murry, supra.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), β glucuronidase and its substrate β -D-glucuronoside, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HPRAP is inserted within a marker gene sequence, transformed cells containing sequences encoding HPRAP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HPRAP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HPRAP and that

express HPRAP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of HPRAP using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HPRAP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN, Section IV; Coligan, J. E. et al. (1997 and periodic supplements) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York, NY; and Maddox, D.E. et al. (1983) *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HPRAP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HPRAP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by APB and Promega (Madison, WI). Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HPRAP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HPRAP may be designed to contain signal sequences which direct secretion of HPRAP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the

inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a “prepro” form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Manassas, VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HPRAP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HPRAP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of HPRAP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HPRAP encoding sequence and the heterologous protein sequence, so that HPRAP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel, supra, ch 10. A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HPRAP may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably ³⁵S-methionine.

Fragments of HPRAP may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra pp. 55-60.)

Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Applied Biosystems). Various fragments of HPRAP may be synthesized separately and then combined to produce the full length molecule.

5 **THERAPEUTICS**

Chemical and structural similarity exists between HPRAP-1 and an endooligopeptidase A related protein from O. cuniculus (GI 2827886). In addition, HPRAP-1 is expressed in cancer and immortalized cell lines and tissues associated with inflammation and the immune response. Therefore, HPRAP-1 appears to play a role in cell proliferative and immune disorders.

10 Chemical and structural similarity exists between HPRAP-2 and the kunitz type protease inhibitor, bikunin, from human (GI 2065529). In addition, HPRAP-2 is expressed in cancer and immortalized cell lines and tissues associated with inflammation and the immune response. Therefore, HPRAP-2 appears to play a role in cell proliferative and immune disorders.

15 Chemical and structural similarity exists between HPRAP-3 and the human proteasome subunit, p27 (GI 2055256). In addition, HPRAP-3 is expressed in cancer and immortalized cell lines and tissues associated with inflammation and the immune response. Therefore, HPRAP-3 appears to play a role in cell proliferative and immune disorders.

20 Chemical and structural similarity exists between HPRAP-4 and a vacuolar aminopeptidase-related protein from C. elegans (GI 529706). In addition, HPRAP-4 is expressed in cancer and immortalized cell lines and tissues associated with inflammation and the immune response. Therefore, HPRAP-4 appears to play a role in cell proliferative and immune disorders.

25 Therefore, in one embodiment, HPRAP-2 or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder. Such disorders can include, but are not limited to, actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

30 In another embodiment, a vector capable of expressing HPRAP-2 or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HPRAP-2 in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of HPRAP-2 may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those listed above.

In another embodiment, HPRAP-2 or a fragment or derivative thereof may be administered to a subject to treat or prevent an immune disorder. Such disorders can include, but are not limited to, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma.

In another embodiment, a vector capable of expressing HPRAP-2 or a fragment or derivative thereof may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified HPRAP-2 in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of HPRAP-2 may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those listed above.

In a further embodiment, an antagonist of HPRAP-1 may be administered to a subject to treat or prevent a cell proliferative disorder. Such a disorder may include, but is not limited to, those discussed above. In one aspect, an antibody which specifically binds HPRAP-1 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical

agent to cells or tissue which express HPRAP-1.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPRAP-1 may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

5 In a further embodiment, an antagonist of HPRAP-3 may be administered to a subject to treat or prevent a cell proliferative disorder. Such a disorder may include, but is not limited to, those discussed above. In one aspect, an antibody which specifically binds HPRAP-3 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HPRAP-3.

10 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPRAP-3 may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

In a further embodiment, an antagonist of HPRAP-4 may be administered to a subject to treat or prevent a cell proliferative disorder. Such a disorder may include, but is not limited to, those discussed above. In one aspect, an antibody which specifically binds HPRAP-4 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HPRAP-4.

15 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPRAP-4 may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

20 In a further embodiment, an antagonist of HPRAP-1 may be administered to a subject to treat or prevent an immune disorder. Such a disorder may include, but is not limited to, those discussed above. In one aspect, an antibody which specifically binds HPRAP-1 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HPRAP-1.

25 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPRAP-1 may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those described above.

30 In a further embodiment, an antagonist of HPRAP-3 may be administered to a subject to treat or prevent an immune disorder. Such a disorder may include, but is not limited to, those discussed above. In one aspect, an antibody which specifically binds HPRAP-3 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HPRAP-3.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPRAP-3 may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those described above.

In a further embodiment, an antagonist of HPRAP-4 may be administered to a subject to treat or prevent an immune disorder. Such a disorder may include, but is not limited to, those discussed above. In one aspect, an antibody which specifically binds HPRAP-4 may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express HPRAP-4.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding HPRAP-4 may be administered to a subject to treat or prevent an immune disorder including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of HPRAP may be produced using methods which are generally known in the art. In particular, purified HPRAP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind HPRAP. Antibodies to HPRAP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with HPRAP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to

HPRAP have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of HPRAP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to HPRAP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc. Natl. Acad. Sci.* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce HPRAP-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) *Proc. Natl. Acad. Sci.* 88:10134-10137.)

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci.* 86: 3833-3837; and Winter, G. et al. (1991) *Nature* 349:293-299.)

Antibody fragments which contain specific binding sites for HPRAP may also be generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) *Science* 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either

polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between HPRAP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering HPRAP epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

In another embodiment of the invention, the polynucleotides encoding HPRAP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding HPRAP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding HPRAP. Thus, complementary molecules or fragments may be used to modulate HPRAP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding HPRAP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding HPRAP. (See, e.g., Sambrook, supra; and Ausubel, supra.)

Genes encoding HPRAP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding HPRAP. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding HPRAP. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using

triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding HPRAP.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HPRAP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable

for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature

5 Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

10 An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of HPRAP, antibodies to HPRAP, and mimetics, agonists, antagonists, or inhibitors of HPRAP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical
15 carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal,
20 enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of
25 Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

30 Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose,

mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic

acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HPRAP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example HPRAP or fragments thereof, antibodies of HPRAP, and agonists, antagonists or inhibitors of HPRAP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic effects is the therapeutic index, and it can be expressed as the LD_{50}/ED_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4

days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind HPRAP may be used for the diagnosis of disorders characterized by expression of HPRAP, or in assays to monitor patients being treated with HPRAP or agonists, antagonists, or inhibitors of HPRAP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for HPRAP include methods which utilize the antibody and a label to detect HPRAP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring HPRAP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of HPRAP expression. Normal or standard values for HPRAP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to HPRAP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of HPRAP expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding HPRAP may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of HPRAP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of HPRAP, and to monitor regulation of HPRAP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide

sequences, including genomic sequences, encoding HPRAP or closely related molecules may be used to identify nucleic acid sequences which encode HPRAP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high,

intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding HPRAP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the HPRAP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequences of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 or from genomic sequences including promoters, enhancers, and introns of the HPRAP gene.

Means for producing specific hybridization probes for DNAs encoding HPRAP include the cloning of polynucleotide sequences encoding HPRAP or HPRAP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding HPRAP may be used for the diagnosis of a disorder associated with expression of HPRAP. Examples of such a disorder include, but are not limited to, cell proliferative disorders such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and immune disorders such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease,

Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma. The polynucleotide sequences encoding HPRAP may be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patients to detect altered HPRAP expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding HPRAP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding HPRAP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding HPRAP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of HPRAP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding HPRAP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from

several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding HPRAP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced *in vitro*. Oligomers will preferably contain a fragment of a polynucleotide encoding HPRAP, or a fragment of a polynucleotide complementary to the polynucleotide encoding HPRAP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of HPRAP include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; and Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) *Proc. Natl. Acad. Sci.* 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) *Proc. Natl. Acad. Sci.* 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding HPRAP may be

used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Price, C.M. (1993) *Blood Rev.* 7:127-134; and Trask, B.J. (1991) *Trends Genet.* 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New York, NY, pp. 965-968.)

Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding HPRAP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, HPRAP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between HPRAP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds

having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with HPRAP, or fragments thereof, and washed. Bound HPRAP is then detected by methods well known in the art. Purified HPRAP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding HPRAP specifically compete with a test compound for binding HPRAP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HPRAP.

In additional embodiments, the nucleotide sequences which encode HPRAP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

EXAMPLES

I. cDNA Library Construction

THP1NOB01

The THP1NOB01 Library was constructed using RNA isolated from cultured, unstimulated THP-1 cells. THP-1 (ATCC TIB 202) is a human promonocyte line derived from the peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia. The THP1NOB01 cDNA library was custom constructed in the Uni-ZAP vector system by Stratagene.

BLADNOT04

The BLADNOT04 Library was constructed using 1 microgram of polyA RNA isolated from bladder tissue of a 28-year-old Caucasian male who died from a self-inflicted gunshot wound (specimen #RA95-09-0677; International Institute of Advanced Medicine, Exton, PA). The tissue donor had a history of smoking and substance abuse.

BEPINOT01

The BEPINOT01 Library was constructed using 1.1 micrograms of polyA RNA isolated from a bronchial epithelium (NHBE) primary cell line derived from a 54-year-old Caucasian male.

OVARNOT03

The OVARNOT03 Library was constructed using 1 microgram of polyA RNA isolated from nontumorous ovarian tissue removed from a 43-year-old Caucasian female during a bilateral salpingo-oophorectomy (removal of the fallopian tubes and ovaries). Family history included atherosclerotic coronary artery disease, cerebrovascular disease, and breast, pancreatic and uterine cancer.

BLADNOT04, BEPINOT01, and OVARNOT03

The frozen tissue was homogenized and lysed using a POLYTRON homogenizer (Brinkmann Instruments, Westbury, NJ) in guanidinium isothiocyanate solution. The lysate was centrifuged over a 5.7 M CsCl cushion using an SW28 rotor (Beckman Coulter, Fullerton CA) in an L8-70M ultracentrifuge (Beckman Coulter) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with acid phenol pH 4.7, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in RNase-free water, and DNase treated for 15 min at 37°C. RNA extraction and precipitation were repeated as before. The mRNA was isolated with the OLIGOTEX kit (Qiagen, Inc., Chatsworth CA) and used to construct the cDNA library.

The mRNAs were handled according to the recommended protocols in the SUPERScript plasmid system (Life Technologies). cDNAs were fractionated on a SEPHAROSE CL4B column (APB), and those cDNAs exceeding 400 bp were ligated into either PSPORTI plasmid (Life Technologies) or pINCY 1 plasmid (Incyte Genomics, Palo Alto CA). The plasmid was subsequently transformed into DH5 α or DH12S competent cells (Life Technologies).

II. Isolation and Sequencing of cDNA Clones

For BLADNOT04 and BEPINOT01, plasmid DNA was released from the cells and purified using the REAL PREP 96 plasmid kit (Qiagen). For OVARNOT03, plasmid DNA was released from cells and purified using the MINIPREP kit (Edge Biosystems, Gaithersburg MD). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile TERRIFIC BROTH (BD Biosciences, Sparks MD) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4°C.

The cDNAs were prepared for sequencing using the MICROLAB 2200 system (Hamilton) in combination with the DNA ENGINE thermal cyclers (MJ Research). The cDNAs were sequenced by the method of Sanger and Coulson (1975; J Mol Biol 94:441-448) using an ABI PRISM 377

sequencing system (Applied Biosystems). Most of the isolates were sequenced according to standard ABI protocols and kits (Applied Biosystems) with solution volumes of 0.25x-1.0x concentrations. In the alternative, cDNAs were sequenced using solutions and dyes from APB.

III. Similarity Searching of cDNA Clones and Their Deduced Proteins

5 BLADNOT04, BEPINOT01, and OVARNOT03

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of similarity using BLAST (Basic Local Alignment Search Tool). (See, e.g., Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; and Altschul et al. (1990) J. Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms could have been used when dealing with primary sequence patterns and secondary structure gap penalties. (See, e.g., Smith, T. et al. (1992) Protein Engineering 5:35-51.) The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at 10^{-25} for nucleotides and 10^{-8} for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and other mammalian sequences (mam), and deduced amino acid sequences from the same clones were then searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp), and eukaryote (eukp), for similarity.

THPINOB01

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT 670 sequence analysis system. In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence,

and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT 670 sequence analysis system (Applied Biosystems) in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

Additionally, sequences identified from cDNA libraries may be analyzed to identify those gene sequences encoding conserved protein motifs using an appropriate analysis program, e.g., BLOCKS. BLOCKS is a weighted matrix analysis algorithm based on short amino acid segments, or blocks, compiled from the PROSITE database. (Bairoch, A. et al. (1997) *Nucleic Acids Res.* 25:217-221.) The BLOCKS algorithm is useful for classifying genes with unknown functions. (Henikoff, S. and Henikoff, G.J. (1991) *Nucleic Acids Research* 19:6565-6572.) Blocks, which are 3-60 amino acids in length, correspond to the most highly conserved regions of proteins. The BLOCKS algorithm compares a query sequence with a weighted scoring matrix of blocks in the BLOCKS database. Blocks in the BLOCKS database are calibrated against protein sequences with known functions from the SWISS-PROT database to determine the stochastic distribution of matches. Similar databases such as PRINTS, a protein fingerprint database, are also searchable using the BLOCKS algorithm. (Attwood, T. K. et al. (1997) *J. Chem. Inf. Comput. Sci.* 37:417-424.) PRINTS is based on non-redundant sequences obtained from sources such as SWISS-PROT, GenBank, PIR, and NRL-3D.

The BLOCKS algorithm searches for matches between a query sequence and the BLOCKS or PRINTS database and evaluates the statistical significance of any matches found. Matches from a BLOCKS or PRINTS search can be evaluated on two levels, local similarity and global similarity. The degree of local similarity is measured by scores, and the extent of global similarity is measured by score ranking and probability values. A score of 1000 or greater for a BLOCKS match of highest ranking indicates that the match falls within the 0.5 percentile level of false positives when the matched block is calibrated against SWISS-PROT. Likewise, a probability value of less than 1.0×10^{-3} indicates that the match would occur by chance no more than one time in every 1000 searches. Only those matches with a cutoff score of 1000 or greater and a cutoff probability value of 1.0×10^{-3} or less are considered in the functional analyses of the protein sequences in the Sequence Listing.

Nucleic and amino acid sequences of the Sequence Listing may also be analyzed using PFAM. PFAM is a Hidden Markov Model (HMM) based protocol useful in protein family searching. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) *Cur. Opin. Str. Biol.* 6:361-365.)

5 The PFAM database contains protein sequences of 527 protein families gathered from publicly available sources, e.g., SWISS-PROT and PROSITE. PFAM searches for well characterized protein domain families using two high-quality alignment routines, seed alignment and full alignment. (See, e.g., Sonnhammer, E.L.L. et al. (1997) *Proteins* 28:405-420.) The seed alignment utilizes the hmms program, a program that searches for local matches, and a non-redundant set of the PFAM database. The full alignment utilizes the hmmsf program, a program that searches for multiple fragments in long sequences, e.g., repeats and motifs, and all sequences in the PFAM database. A result or score of 100 "bits" can signify that it is 2^{100} -fold more likely that the sequence is a true match to the model or comparison sequence. Cutoff scores which range from 10 to 50 bits are generally used for individual protein families using the SWISS-PROT sequences as model or comparison sequences.

15 Two other algorithms, SIGPEPT and TM, both based on the HMM algorithm described above (see, e.g., Eddy, supra; and Sonnhammer, supra), identify potential signal sequences and transmembrane domains, respectively. SIGPEPT was created using protein sequences having signal sequence annotations derived from SWISS-PROT. It contains about 1413 non-redundant signal sequences ranging in length from 14 to 36 amino acid residues. TM was created similarly using transmembrane domain annotations. It contains about 453 non-redundant transmembrane sequences encompassing 1579 transmembrane domain segments. Suitable HMM models were constructed using the above sequences and were refined with known SWISS-PROT signal peptide sequences or transmembrane domain sequences until a high correlation coefficient, a measurement of the correctness of the analysis, was obtained. Using the protein sequences from the SWISS-PROT database as a test set, a cutoff score of 11 bits, as determined above, correlated with 91-94% true-positives and about 4.1% false-positives, yielding a correlation coefficient of about 0.87-0.90 for SIGPEPT. A score of 11 bits for TM will typically give the following results: 75% true positives; 1.72% false positives; and a correlation coefficient of 0.76. Each search evaluates the statistical significance of any matches found and reports only those matches that score at least 11 bits.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a

gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; and Ausubel, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ database (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar.

The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of Northern analysis are reported as a list of libraries in which the transcript encoding HPRAP occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V. Extension of HPRAP Encoding Polynucleotides

The nucleic acid sequences of Incyte Clones 031381, 1319265, 2057812, and 2058485 were used to design oligonucleotide primers for extending partial nucleotide sequences to full length. For each nucleic acid sequence, one primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO software (MBI), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (Life Technologies) were used to extend the sequence. If

more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Applied Biosystems) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the DNA ENGINE thermal cycler (MJ Research), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
5	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
10	Step 7	Repeat steps 4 through 6 for an additional 15 cycles
	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
15	Step 10	68° C for 7:15 min
	Step 11	Repeat steps 8 through 10 for an additional 12 cycles
	Step 12	72° C for 8 min
20	Step 13	4° C (and holding)

A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using a QIAQUICK kit (Qiagen), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) were transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium. (See, e.g., Sambrook, *supra*, Appendix A, p. 2.) After incubation for one hour at 37° C, the *E. coli* mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, *supra*, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150 μ l of liquid LB/2x carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5 μ l from each sample was transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the

extension reaction were added to each well. Amplification was performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2 through 4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO software (MBI) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ - 32 P] adenosine triphosphate (APB), and T4 polynucleotide kinase (NEN Life Science Products, Inc., Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (APB). An aliquot containing 10⁷ counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, XbaI, or Pvu II (NEN Life Science Products).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (NYTRAN PLUS, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR film (Eastman Kodak, Rochester, NY) is exposed to the blots, hybridization patterns are compared visually.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) *Science* 270:467-470; and Shalon, D. et al. (1996) *Genome Res.* 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the HPRAP-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring HPRAP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO software (MBI) and the coding sequence of HPRAP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the HPRAP-encoding transcript.

IX. Expression of HPRAP

Expression and purification of HPRAP is achieved using bacterial or virus-based expression systems. For expression of HPRAP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA

transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3).

Antibiotic resistant bacteria express HPRAP upon induction with isopropyl beta-D-

thiogalactopyranoside (IPTG). Expression of HPRAP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding HPRAP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, HPRAP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (APB).

Following purification, the GST moiety can be proteolytically cleaved from HPRAP at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (Qiagen). Methods for protein expression and purification are discussed in Ausubel, supra, ch 10, 16. Purified HPRAP obtained by these methods can be used directly in the following activity assay.

X. Demonstration of HPRAP Activity

The protease activity of HPRAP-1 and HPRAP-4 is measured by the hydrolysis of appropriate synthetic peptide substrates conjugated with various chromogenic molecules in which the degree of hydrolysis is quantitated by spectrophotometric (or fluorometric) absorption of the released chromophore. (Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York, NY, pp.25-55.) Peptide substrates are designed according to the category of protease activity as endopeptidase (serine, cysteine, aspartic proteases), aminopeptidase (leucine aminopeptidase), or carboxypeptidase (carboxypeptidase A and B, procollagen C-

proteinas). Chromogens commonly used are 2-naphthylamine, 4-nitroaniline, and furylacrylic acid. Assays are performed at ambient temperature and contain an aliquot of the enzyme and the appropriate substrate in a suitable buffer. Reactions are carried out in an optical cuvette and followed by the increase/decrease in absorbance of the chromogen released during hydrolysis of the peptide substrate. The change in absorbance is proportional to the enzyme activity in the assay.

The assay for HPRAP-3 is carried out as described above using as the source of protease activity, a proteasome complex reconstituted with HPRAP-3 in the absence of the p27 subunit.

The assay for HPRAP-2 is carried out as described above for HPRAP-1 and HPRAP-4 using a serine protease assayed in the absence and in the presence of various concentrations of HPRAP-2.

Inhibition of serine protease activity is proportional to the activity of HPRAP-2 in the assay.

XI. Functional Assays

HPRAP function is assessed by expressing the sequences encoding HPRAP at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include PCMV SPORT plasmid (Life Technologies) and PCR 3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μ g of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2 μ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., GFP (Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate properties, for example, their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York, NY.

The influence of HPRAP on gene expression can be assessed using highly purified

populations of cells transfected with sequences encoding HPRAP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (Dyna, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding HPRAP and other genes of interest can be analyzed by Northern analysis or microarray techniques.

XII. Production of HPRAP Specific Antibodies

HPRAP substantially purified using polyacrylamide gel electrophoresis (PAGE) (see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the HPRAP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel *supra*, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using fmoc-chemistry and coupled to KLH (Sigma Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for anti-peptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIII. Purification of Naturally Occurring HPRAP Using Specific Antibodies

Naturally occurring or recombinant HPRAP is substantially purified by immunoaffinity chromatography using antibodies specific for HPRAP. An immunoaffinity column is constructed by covalently coupling anti-HPRAP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE resin (APB). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HPRAP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HPRAP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HPRAP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such

as urea or thiocyanate ion), and HPRAP is collected.

XIV. Identification of Molecules Which Interact with HPRAP

5 HPRAP, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled HPRAP, washed, and any wells with labeled HPRAP complex are assayed. Data obtained using different concentrations of HPRAP are used to calculate values for the number, affinity, and association of HPRAP with the candidate molecules.

10 Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

15

What is claimed is:

1. An isolated polynucleotide encoding a protein selected from:
 - a) an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4;
 - b) a variant having at least 90% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4;
 - c) an antigenic epitope of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4;
 - d) an oligopeptide of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4; and
 - e) a biologically active portion of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.
2. An isolated polynucleotide or the complement thereof selected from:
 - a) a polynucleotide selected from the group consisting of SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8;
 - b) a fragment of SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8;
 - c) a variant having at least 70% identity to the nucleic acid sequence of SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8; and
 - d) an oligonucleotide of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8.
3. A composition comprising the polynucleotide or the complement of the polynucleotide of claim 1.
4. A substrate comprising the polynucleotide or the complement of the polynucleotide of claim 1.
5. A probe comprising the polynucleotide or the complement of the polynucleotide of claim 1.
6. A vector comprising the polynucleotide of claim 1.
7. A host cell comprising the vector of claim 6.
8. A method for producing a protein, the method comprising:
 - a) culturing the host cell of claim 7 under conditions for protein expression; and
 - b) recovering the protein from the host cell culture.
9. A transgenic cell line or organism comprising the vector of claim 6.
10. A method for using a polynucleotide to detect the differential expression of a nucleic acid in a sample comprising:

a) hybridizing the probe of claim 5 to the nucleic acids, thereby forming hybridization complexes; and

b) comparing hybridization complex formation with a standard, wherein the comparison indicates the differential expression of the polynucleotide in the sample.

11. The method of claim 10 further comprising amplifying the nucleic acids of the sample prior to hybridization.

12. A method of using a polynucleotide to screen a plurality of molecules or compounds, the method comprising:

a) combining the polynucleotide of claim 1 with a plurality of molecules or compounds under conditions to allow specific binding; and

b) detecting specific binding, thereby identifying a molecule or compound which specifically binds the polynucleotide.

13. The method of claim 12 wherein the molecules or compounds are selected from DNA molecules, RNA molecules, peptide nucleic acids, artificial chromosome constructions, peptides, transcription factors, repressors, and regulatory molecules.

14. A purified protein selected from:

a) an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4;

b) a variant having at least 90% identity to the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4;

c) an antigenic epitope of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4;

d) an oligopeptide of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4; and

e) a biologically active portion of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

15. A composition comprising the protein of claim 14.

16. A method for using a protein to screen a plurality of molecules or compounds to identify at least one ligand, the method comprising:

a) combining the protein of claim 14 with the molecules or compounds under conditions to allow specific binding; and

b) detecting specific binding, thereby identifying a ligand which specifically binds

the protein.

17. The method of claim 16 wherein the molecules or compounds are selected from DNA molecules, RNA molecules, peptide nucleic acids, peptides, proteins, mimetics, agonists, antagonists, antibodies, immunoglobulins, inhibitors, and drugs.

- 5 18. A method of using a mammalian protein to prepare and purify antibodies comprising:
- a) immunizing a animal with the protein of claim 14 under conditions to elicit an antibody response;
 - b) isolating animal antibodies;
 - c) attaching the protein to a substrate;
 - 10 d) contacting the substrate with isolated antibodies under conditions to allow specific binding to the protein;
 - e) dissociating the antibodies from the protein, thereby obtaining purified antibodies.
- 15 19. A purified antibody comprising an antibody produced by the method of claim 18.

ABSTRACT OF THE DISCLOSURE

5 The invention provides human protease associated proteins (HPRAP) and polynucleotides which identify and encode HPRAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating or preventing disorders associated with expression of HPRAP.

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By: Nancy RamosPrinted: Nancy Ramos

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hillman et al.

Title: HUMAN PROTEASE ASSOCIATED PROTEINS

Serial No.: To Be Assigned

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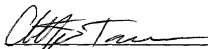
Transmitted herewith are Figures 1A, 1B, 1C, 1D, 1E, 1F, 2A, 2B, 2C, 2D, 2E 3A, 3B, 3C, 4A, 4B, 4C, 4D, 4E, 5A, 5B, 5C, 5D, 6A, 6B, 7A, 7B, 8A, 8B, and 8C as thirty (30) sheets of formal drawings for this application. Each sheet of drawing indicates the identifying indicia suggested in 37 CFR Section 1.84(c) on the reverse side of the drawings.

Applicants believe that no fee is due with this paper. However, if the Commissioner determines that a fee is necessary, the Commissioner is hereby authorized to charge any additional fees associated with this communication or credit any overpayment to Deposit Account No. 09-0108. **A duplicate copy of this communication is enclosed.**

If there are any questions regarding the above, the Examiner is invited to call the undersigned at 650-855-0555.

Respectfully submitted,

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11	20	29	38	47	56
5' CGGGC GCG GAG GTA CGC TGA GTG GAG CTC GGG GCT TAG GCG AGC TGA GCC GAG					
65	74	83	92	101	110
CGG CTG GGC GGG CCT GGC CGG GCC AGC GGA GGG GAG ACG TCG GTT GAG CGG CGG					
119	128	137	146	155	164
CGA ACA TGC GCT TTT GAC ACA TTG GAG GCT TTC TTG ATC ATG GAT GGT GAA GAT					
				M D G E D	
173	182	191	200	209	218
ATA CCA GAT TTT TCA AGT TTA AAG GAG GAA ACT GCT TAT TGG AAG GAA CTT TCC					
I P D F S S L K E E T A Y W K E L S					
227	236	245	254	263	272
TTG AAG TAT AAG CAA AGC TTC CAG GAA GCT CGG GAT GAG CTA GTT GAA TTC CAG					
L K Y K Q S F Q E A R D E L V E F Q					
281	290	299	308	317	326
GAA GGA AGC AGA GAA TTA GAA GCA GAG TTG GAG GCA CAA TTA GTA CAG GCT GAA					
E G S R E L E A E L E A Q L V Q A E					
335	344	353	362	371	380
CAA AGA AAT AGA GAC TTG CAG GCT GAT AAC CAA AGA CTG AAA TAT GAA GTG GAG					
Q R N R D L Q A D N Q R L K Y E V E					

FIGURE 1A

389 GCA TTA AAG GAG AAG CTA GAG CAT CAA TAT GCA CAG AGC TAT AAG CAG GTC TCA 434
A L K E K L E H Q Y A Q S Y K Q V S

443 GTG TTA GAA GAT GAT TTA AGT CAG ACT CGG GCC AIT AAG GAG CAG TTG CAT AAG 488
V L E D L S Q T R A I K E Q L H K

497 TAT GTG AGA GAG CTG GAG CAG GCC AAC GAC GAC CTG GAG CGA GCC AAA AGG GCA 542
Y V R E L E Q A N D L E R A K R A

551 ACA ATA GTT TCA CTG GAA GAC TTT GAA CAA AGG CTA AAC CAG GCC ATT GAA CGA 596
T I V S L E S L E D F E Q R L N Q A I E R

605 AAT GCA TTT TTA GAA AGT GAA CTT GAT GAA AAG GAA TCT TTG GTC TCT GTA 650
N A F L E S E L D E K E S L L V S V

659 CAG AGG TTA AAG GAT GAA GCA AGA GAT TTA AGG CAA GAA CTA GCA GTT CGG GAA 704
Q R L K D E A R D L R Q E L A V R E

713 AGA CAA CAG GAA GTA ACT AGA AAG TCG GCT CCT AGC TCT CCA ACT CTA GAC TGT 758
R Q E V T R K S A P S S P T L D C

FIGURE 1B

767	GAA AAG ATG GAC TCC GCC GTC CAA GCA TCA CTT TCT TTG CCA GCT ACC CCT GTT	776	785	794	803	812
E K M D S A V Q A S L S L P A T P V						
821	GGC AAA GGA ACG GAG AAC ACT TTT CCT TCA CCG AAA GGT ATA CCA AAT GGT TTT	830	839	848	857	866
G K G T E N T F P S P K A I P N G F						
875	GGT ACC AGT CCA CTA ACT CCC TCT GCT AGG ATA TCA GCA CTA AAC ATC GTG GGG	884	893	902	911	920
G T S P L T P S A R I S A L N I V G						
929	GAT CTC TTA CGG AAA GTA GGG GCT TTA GAA TCC AAA TTA GCA GCT TGC AGG AAT	938	947	956	965	974
D L L R K V G A L E S K L A A C R N						
983	TTT GCA AAG GAC CAA GCA TCA CGA AAA TCC TAT ATT TCA GGG AAT GTT AAC TGT	992	1001	1010	1019	1028
F A K D Q A S R K S Y I S G N V N C						
1037	GGG GTG CTG AAT GGC AAT GGC ACA AAG TTC TCT CGA TCA GGG CAT ACA TCT TTC	1046	1055	1064	1073	1082
G V L N G N G T K F S R S G H T S F						
1091	TTC GAC AAA GGG GCA GTA AAC GGC TTT GAC CCC GCT CCT CCT CCT GGT CTG	1100	1109	1118	1127	1136
F D K G A V N G F D P A P P P G L						

FIGURE 1C

1145	1154	1163	1172	1181	1190
GGC TCC TCG CGT CCA TCG TCA GCG CCG GGT ATG CTG CCT CTC AGT GTG CGA GTG					
G S S R P S S A P G M L P L S V R V					
1199	1208	1217	1226	1235	1244
CCT AGC CTC CAG GTG GGG GCT CCT GCC CTC CTC CAA CAA CCC AGG ACA CCC ACG					
P S L Q V G A P A L L Q Q P R T T					
1253	1262	1271	1280	1289	1298
CCT CAC CCC TCG GTG CCT GGG CCC AGC CCC GTG CCC CTC CGT CTG CCT CCG CAC					
P H P S V P G P S P V P L R L P P H					
1307	1316	1325	1334	1343	1352
GGC TGG CAG AGG GCA GGC TGC ATG CAG TGG CGG CTA CTG GGC CCT GCC CAG CCC					
G W Q R A G C M Q W R L L G P A Q P					
1361	1370	1379	1388	1397	1406
CGG AAC TCT GCG CGA TAT CAA TAC TGG CTA TTT TCT CTT CTC GCC GTA GTG CCG					
R N S A R Y Q Y W L F S L L A V V P					
1415	1424	1433	1442	1451	1460
TTG GTT TCA CAT GAT TGC ACT TTT GTG GGT CGC AAG GTG ATA CAT ACG TGT ATT					
L V S H D C T F V G R K V I H T C I					
1469	1478	1487	1496	1505	1514
ACT TGG TCA CTG GAT GCA GAA GTA CCC ATT CAT CAC ACC TGC CCC ATA GCC CCC					
T W S L D A E V P I H H T C P I A P					

FIGURE 1D

1523	1532	1541	1550	1559	1568
ACT CTG CTG TAC TGA TAG GAT TTA GTT GTG TTT TAG GAC AAT GCA AAT CTT CTA					
T L L Y					
1577	1586	1595	1604	1613	1622
GAA GTT CTC CCC CAA ATC AGG TCA ATG TGT GCC CTC CTG AGC TCC CAC CCA GGC					
1631	1640	1649	1658	1667	1676
ATC TCC AGT GCT CAT GAT CAT GTG TCC CCC AAC TCC ACC CCT CAC AGT TTG GGC					
1685	1694	1703	1712	1721	1730
CTG TTT CTG GCA AAG AGT CAG GAA GGT TAC TGA ATT AGG GAA CAT TTT CTG CAC					
1739	1748	1757	1766	1775	1784
CTT CTG ATT TTA CTT AAG CAG CTA CCA TTC CAT GGA CTT GCC TCC CAG AGC AGC					
1793	1802	1811	1820	1829	1838
ACA ATG CCC GTC TGA GCC CCA CGT GGC AGG AGC CTC TGG GAC GGC GCA CAC ACA					
1847	1856	1865	1874	1883	1892
GGC CCA GCC TCT GTG CTG TCT CCT CCT CTG TGC GCC TCA GAC TCG GGC TGA GGC					
1901	1910	1919	1928	1937	1946
AGG CGG GCA GCC TCT CGC CAG CCT TCC CGT CCT TCA GTT CAA CGA CAT CTT TGG					

FIGURE 1E

1955	1964	1973	1982	1991	2000
AGT GTT TTT	GTT TTC TCT	TCC AAG GGC	CGT CCC GTT	GTG TTA GGA	AGG GTG AGT
2009	2018	2027	2036	2045	2054
GGC TGG TTC	CAG GGT GGG	CCG GTG CCA	GCT CCG GGG	TGG ACT GAA	CAG CGG CGG
G W F Q	G G P	P V P	A P G	W T E	Q R R
2063	2072	2081	2090	2099	2108
CTG TCC CTG	TGC ATC CTT	TGA TTA CTC	TCA TGC TGC	ATT TAC TGT	TTA CAT TTG
2117	2126	2135	2144	2153	2162
TTT TAT TGT	ACA TAG GTT	TGT AAA CAT	TAT TGC CTG	AGA TAT TTG	TAT ATA ACT
2171	2180	2189	2198	2207	2216
TGG GCT TTG	TAG CTT TTA	TTT ATT CAG	AAC GCA TAC	GGC ATG TTA	ATG ACT CTG
2225	2234	2243	2252	2261	2270
ATG GTG TCC	TCC TCT GGG	CAG CTG TAT	AGG ATC ATC	ATG TGG TTA	CAA AAA ATA
2279	2288	2297	2306	2315	2324
CTT CCC TCA	AAA AAA TTC	TTT TAA TGT	GGA AAC AAT	AAA TTT CAC	AGA AAA AAA
AAA	3'				

FIGURE 1F

	10	19	28	37	46	55
5' CTGA GAG AAG CCT GGT CCA TCT AGT GAG AAT TGA CCT TAT CTC ACT TTC TCT CCC						
	64	73	82	91	100	109
CGC CAG GGT CTG GGA TCC CCA AGG CCT GGG CAG GCA TAG ACT TGA AGG TAC AAC						
	118	127	136	145	154	163
CCC AGG AAC CCC TGG TGC TGA AGG ATG M W K T Q I G A Y C						
	172	181	190	199	208	217
GGG GTG ACA ACG GAT GTC AGG GTA GAG AGG AAA GAC CCA AAC CAG GTG GAA CTG G V T T D V R V E R K D P N Q V E L						
	226	235	244	253	262	271
TGG GGA CTC AAG GAA GGC ACC TAC CTG TTC CAG CTG ACA GTG ACT AGC TCA GAC W G L K E G T Y L F Q L T V T S S D						
	280	289	298	307	316	325
CAC CCA GAG GAC ACG GCC AAC GTC ACA GTC ACT GTG CTG TCC ACC AAG CAG ACA H P E D T A N V T V T V L S T K Q T						
	334	343	352	361	370	379
GAA GAC TAC TGC CTC GCA TCC AAC AAG GTG GGT CGC CGC TGC CGG GGC TCCT TTTC E D Y C L A S N K V G R R C R G S F						

—

388	CGG CGC TGG TAC TAT GAC CCC ACG GAG CAG ATC TGC AAG AGT TTC GTT TAT GGA	406	415	424	433
	P R W Y Y D P T E Q I C K S F V Y G				
442	GGC TGC TTG GGC AAC AAG AAC AAC TAC CTT CGG GAA GAA GAG TGC ATT CTA GCC	451	460	478	487
	G C L G N K N N Y L R E E C I L A				
496	TGT CGG GGT GTG CAA GGT GGG CCT TTG AGA GGC AGC TTCT GGG GCT CAG GCG ACT	505	514	532	541
	C R G V Q G G P L R G S S G A Q A T				
550	TTC CCC CAG GGC CCC TCC ATG GAA AGG CGC CAT CCA GTG TGC TCT GGC ACC TGT	559	568	577	595
	F P Q G G P S M E R R H P V C S G T C				
604	CAG CCC ACC CAG TTC CGC TGC AGC AAT GGC TGC ATC GAC AGT TTC CTG GAG	613	622	631	649
	Q P T Q F R C S N G C C I D S F L E				
658	TGT GAC GAC ACC CCC AAC TGC CCC GAC GCC TCC GAC GAG GCT GCC TGT GAA AAA	667	676	685	703
	C D D T P N C P D A S D E A A C E K				
712	TAC AGG AGT GGC TTT GAC GAG CTC CAG CGC ATC CAT TTC CCC AGT GAC AAA GGG	721	730	739	757
	Y T S G F D E L Q R I H F P S D K G				

FIGURE2B

766	CAC TGC GTG GAC CTG CCA	775	784	793	802	811
H C V D L P D T G L C K E S I P R W						
820	TAC AAC CCC TTC AGC GAA CAC TGC GCC CGC TTT ACC TAT GGT TGT TAT	829	838	847	856	865
Y Y N P F S E H C A R F T Y G G C Y						
874	GGC AAC AAG AAC AAC TTT GAG GAA GAG CAG TGC CTC GAG TCT TGT CGC GGC	883	892	901	910	919
G N K N F E E E Q Q C L E S C R G						
928	ATC TCC AAG AAG GAT GTG TTT GGC CTG AGG CGG GAA ATC CCC ATT CCC AGC ACA	937	946	955	964	973
I S K K D V F G L R R E I P I P S T						
982	GGC TCT GTG GAG ATG GCT GTC GCA GTG TTC CTG GTC ATC TGC ATT GTG GTG GTG	991	1000	1009	1018	1027
G S V E M A V A V F L V I C I V V V						
1036	GTA GCC ATC TTG GGT TAC TGC TTC TTC AAG AAC CAG AGA AAG GAC TTC CAC GGA	1045	1054	1063	1072	1081
V A I L G Y C F F K N Q R K D F H G						
1090	CAC CAC CAC CAC CCA CCA CCC ACC CCT GCC AGC TCC ACT GTC TCC ACT ACC GAG	1099	1108	1117	1126	1135
H H H H P P P T P A S S T V S T T E						

FIGURE2C

1144	GAC AGG GAG CAC CTG GTC TAT AAC CAC ACC ACC CGG CCC CTC TGA GCC TGG GTC	1153	1162	1171	1180	1189
D T E H L V Y N H T T R P L						
1198	TCA CCG GCT CTC ACC TGG CCC TGC TTC CTG CTT GCC AAG GCA GAG GCC TGG GCT	1207	1216	1225	1234	1243
1252	GGG AAA AAC TTT GGA ACC AGA CTC TTG CCT GTT TCC CAG GCC CAC TGT GCC TCA	1261	1270	1279	1288	1297
1306	GAG ACC AGG GCT CCA GCC CCT CTT GGA GAA GTC TCA GCT AAG CTC ACG TCC TGA	1315	1324	1333	1342	1351
1360	GAA AGC TCA AAG GTT TGG AAG GAG CAG AAA ACC CTT GGG CCA GAA GTA CCA GAC	1369	1378	1387	1396	1405
1414	TAG ATG GAC CTG CCT GCA TAG GAG TTT GGA GGA AGT TGG AGT TTT GTT TCC TCT	1423	1432	1441	1450	1459
1468	GTT CAA AGC TGC CTG TCC CTA CCC CAT GGT GCT AGG AAG AGT GGG GTG GTG	1477	1486	1495	1504	1513

FIGURE 2D

1522	1531	1540	1549	1558	1567
TCA GAC CCT GGA	GGC CCC AAC	CCT GTC CTC	CCG AGC TCC	TCT TCC ATG	CTG TGC
1576	1585	1594	1603	1612	1621
GCC CAG GGC TGG	GAG GAA GGA	CTT CCC TGT	GTA GTT TGT	GTA AAG AGT	TGC
1630	1639	1648	1657	1666	1675
TTT TTG TTT ATT	TAA TGC TGT	GGC ATG GGT	GAA GAG GAG	GGG AAG AGG	CCT GTT
1684	1693	1702	1711	1720	1729
TGG CCT CTC TGT	CCT CTC TTC	CTC TTC CCC	CAA GAT TGA	GCT CTC TGC	CCT TGA
1738	1747	1756	1765	1774	1783
TCA GCC CCA CCC	TGG CCT AGA	CCA GCA GAC	AGA GCC AGG	AGA GGC TCA	GCT GCA
1792	1801	1810	1819	1828	1837
TTC CGC AGC CCC	CAC CCC CAA	GGT TCT CCA	ACA TCA CAG	CCC AGC CCA	CCC ACT
1846	1855	1864			
GGG TAA TAA AAG	TGG TTT GTG	GAA AAA AAA	AAA AAA	3'	

FIGURE 2E

10	19	28	37	46	55
5' GTTT TGG CGC ATG GGC GGA GCG TAG TTA CGG TCG ACT GGG GCG TCG TCC CTA GCC					
64	73	82	91	100	109
CGG GAG CCG GGT CTC TGG AGT CGC GGC CCG TTC ACG ATG TCC GAC GAG GAA					
				M S D E	
118	127	136	145	154	163
CGC AGG CAG AGC GGC TCC TCG CAG GCC GGC GTG ACT GTC AGC GAC GTC					
A R Q S G G S S Q A G A V T V S D V					
172	181	190	199	208	217
CAG GAG CTG ATG CGC AAG GAG GAG ATA GAA GCG CAG ATC AAG GCC AAC TAT					
Q E L M R R K E E I E A Q I K A N Y					
226	235	244	253	262	271
GAC GTG CTG GAA AGC CAA AAA GGC ATT GGG ATG AAC GAG CCG CTG GTG GAC TGT					
D V L E S Q K G I G M N E P L V D C					
280	289	298	307	316	325
GAG GGC TAC CCC CGG TCA GAC GTG GAC CTG TAC CAA GTC CGC ACC GCC AGG CAC					
E G Y P R S D V D L Y Q V R T A R H					
334	343	352	361	370	379
AAC ATC ATA TGC CTG CAG AAT GAT CAC AAG GCA GTG ATG AAG CAG GTG GAG GAG					
N I I C L Q N D H K A V M K Q V E E					

FIGURE 3A

388	GCC CTG CAC CAG CTG CAC GCT CGC GAC AAG GAG AAG CAG GCC CGG GAC ATG GCT	406	415	424	433
A L H Q	L H A R D K E K Q A R D M A				
442	GAG GCC CAC AAA GAG GCC ATG AGC CGC AAA CTG GGT CAG AGT GAG AGC CAG GGC	460	469	478	487
E A H K	E A M S R K L G Q S E S Q G				
496	CCT CCA CGG GCC TTC GCC AAA GTG AAC AGC ATC AGC CCC GGC TTC CCA GCC AGC	514	523	532	541
P P R A	F A K V N S I S P G S P A S				
550	ATC GCG GGT AAT CCA GCG GTT GGC CAC TCA AGT CCA TGC CCA GGG GAC ACG GGT	568	577	586	595
I A G N	P G V G H S S P C P G D T G				
604	CTG CAA GTG GAT GAT GAT ATT GAT GAG TTC GGC TCT GTG AAC ACC CAG AAC TTC	622	631	640	649
L Q V D	D E I V E F G S V N T Q N F				
658	CAG TCA CTG CAT AAC ATT GGC AGT GTG GTG CAG CAC AGT GAG GGG AAG CCC CTG	676	685	694	703
Q S L H	N I G S V V Q H S E G K P L				
712	AAT GTG ACA GTG ATC CGC AGG GGG GGA AAA CAC CAG CTTT AGA CTTT CCA ACA	730	739	748	757
N V T V	I R R G G K H Q L R L V P T				

FIGURE3B

766	775	784	793	802	811
CGC TGG GCA GGA AAA GGA CTG CTG GGC TGC AAC ATT ATT CCT CTG CAA AGA TGA					
R W A G G K G L L G C N I I P L Q R					
820	829	838	847	856	865
TTG TCC CTG GGG AAC AGT AAC AGG AAA GCA TCT TCC CTT GCC CTG GAC TTG GGT					
874	883	892	901	910	919
CTA GGG ATT TCC AAC TTG TCT TCT CTG CCT GAA GCA TAA GGA TCT GGA AGA GGC					
928	937	946	955	964	973
TTG TAA CCT GAA CTT CTG TGT GGT GGC AGT ACT GTG GCC CAC CAG TGT AAT CTC					
982	991	1000	1009	1018	1027
CCT GGA TTA AGG CAT TCT TAA AAA CTT AGG CTT GGC CTC TTT CAC AAA TTA GGC					
1036	1045	1054	1063	1072	1081
CAC GGC CCT AAA TAG GAA TTC CCT GGA TTG TGG GCA AGT GGG CGG AAG TTA TTC					
1090	1099	1108	1117	1126	1135
TGG CAG GTA CTG GTG TGA TTA TTA TTA TTA TTA ATA AAG AGT TTT ACA GTG					
CTG	3'				

FIGURE3C

5' CGAG CCC GGA	10	19	28	37	46	55
		CAG ATG AGC GGA CAC	AGC CCC AGC CGC GGC	ATG CAG GTA		
AGT GGC TCC CGA	64	73	82	91	100	109
		CGG CCC CAC TTG AAT TTC	GAT CCC AGA CCG	GGT CCG GCG CCC		
TCC GGG GCC CAA	118	127	136	145	154	163
		GCT TAG CGC GGT GCT GCA	GTG GGG CCG CCG	GAC CCA AAG CGA		
AAC CGA AAG CCC	172	181	190	199	208	217
		CGC GGA GGG TGA CCT GAC	GAC TTT CCC GGG	ACT GGA AGG GGG		
AGT CCT GCG AGA	226	235	244	253	262	271
		GAC TAG GTG GCC ATG AAC	GGT AAG GCC CGC AAA	GAG GCG GTG		
			M N G K A R K E A V			
CAG ACT GCG GCT	280	289	298	307	316	325
		AAG GAA CTC CTC AAG	TTT AAC CGG AGT CCC	TCT CCT TTC		
Q T A A K E L L K F V N R S P F						
CAT GCT GTG GCT	334	343	352	361	370	379
		GAA TGC CGC AAC CGC	CTT CTC CAG GCT GGC	TTC AGT GAA CTC		
H A V A E C R N R L L Q A G F S E L						

FIGURE 4A

388	AAG GAG ACT GAG AAA TGG AAT ATT AAG CCC GAG AGC AAG TAC TTC ATG ACC AGG	406	415	424	433
	K E T E K K W N I K K P E S K Y F M T R				
442	AAC TCC TCC ACC ATC ATA GCT TTT GCT GTA GGG GGC CAG TAC GTT CCT GGC AAT	451	460	478	487
	N S S T I I A F A V G G G Q Y V P G N				
496	GGC TTC AGC CTC ATC GGG GCC CAC ACG GAC AGC CCC TGC CTC CGG GTG AAA CGT	505	514	523	541
	G F S L I G A H T D S P C L R V K R				
550	CGG TCT CGC CGC AGC CAG GTG GGC TTC CAG CAA GTC GGT GTG GAG ACC TAT GGT	559	568	577	595
	R S R R S Q V G F Q Q V G V E T Y G				
604	GGT GGG ATC TGG AGC ACC TGG TTT GAC CGT GAC CTG ACT CTG GCT CGA CGC GTG	613	622	631	649
	G G I W S T W F D R D L T L A G R V				
658	ATT GTC AAG TGC CCT ACC TCA GGT CGG CTG GAG CAG CTG GTG CAC GTG GAG	667	676	685	703
	I V K C P T S G R L E Q Q L V H V E				
712	CGG CCC ATT CTT CGC ATC CCA CAC CTG GCC ATC CAT CTG CAG CGA AAT ATC AAC	721	730	739	757
	R P I L R I P H L A I H L Q R N I N				

FIGURE 4B

766	GAG AAC TTTT GGG CCC AAC ACA GAG ATG CAT CTA GTC CCC ATT CTT GCC ACA GCC	775	784	793	802	811
E N F G P N T E M H L V P I L A T A						
820	ATC CAG GAG GAG CTG GAG AAG GGG ACT CCT GAG CCA GGG CCT CTC AAT GCT GTG	829	838	847	856	865
I Q E E L E K G T P E P G P L N A V						
874	GAT GAG CGG CAC CAT TCG GTC CTC ATG TCC CTG CTC TGT GCC CAT CTG GGG CTG	883	892	901	910	919
D E R H H S V L M S L L C A H L G L						
928	AGC CCC AAG GAC ATA GTG GAG ATG GAG CTC TGC CTT GCA GAC ACC CAG CCT GCG	937	946	955	964	973
S P K D I V E M E L C L A D T Q P A						
982	GTC TTG GGT GGT GCC TAT GAT GAG TTC ATC TTT GCT CCT CGG CTG GAC AAT CTG	991	1000	1009	1018	1027
V L G G A Y D E F I F A P R L D N L						
1036	CAC AGC TGC TTC TGT GCC CTG CAG GCC TTG ATA GAT TCC TGT GCA GGC CCT GGC	1045	1054	1063	1072	1081
H S C F C A L Q A L I D S C A G P G						
1090	TCC CTG GCC ACA GAG CCT CAC GTG CGC ATG GTC ACA CTC TTAT GAC AAC GAA GAG	1099	1108	1117	1126	1135
S L A T E P H V R M V T L Y D N E E						

FIGURE 4C

1144	1153	1162	1171	1180	1189
GTG GGG TCT GAG AGT GCA CAG GGA GCA CAG TCA CTG CTG ACA GAG CTG GTG CTG					
V G S E S A Q G A Q A Q S L L T E L V L					
1198	1207	1216	1225	1234	1243
CGG CGG ATC TCA GCC TCG TGC CAG CAC CCG ACA GCC TTC GAG GAA GCC ATA CCC					
R R I S A S C Q H P T A F E E A I P					
1252	1261	1270	1279	1288	1297
AAG TCC TTC ATG ATC AGC GCA GAC ATG GCC CAT GCT GTG CAT CCC AAC TAC CTG					
K S F M I S A D M A H A V H P N Y L					
1306	1315	1324	1333	1342	1351
GAC AAG CAT GAG GAG AAC CAC CGG CCT TTA TTC CAC AAG GGC CCC GTG ATC AAG					
D K H E E N H R P L F H K G P V I K					
1360	1369	1378	1387	1396	1405
GTG AAC AGC AAG CAA CGC TAT GCT TCA AAC CGG GTG TCA GAG GCC CTG ATC CGA					
V N S K Q R Y A S N A V S E A L I R					
1414	1423	1432	1441	1450	1459
GAG GTG GCC AAC AAA GTC AAG GTC CCC CTG CAG GAT CTC ATG GTC CGG AAT GAC					
E V A N K V K V P L Q D L M V R N D					
1468	1477	1486	1495	1504	1513
ACC CCC TGT GGA ACC ACC ATT GGA CCT ATC TTG GCT TCT CGG CTG GGG CTG CGG					
T P C G T T I G P I L A S R L G L R					

FIGURE 4D

1522	GTG	CTG	GAT	TTA	GGC	AGC	CCC	CAA	CTG	GCC	ATG	CAC	TCT	ATC	CGG	GAG	ATG	GCC
	V	L	D	L	G	S	P	Q	L	A	M	H	S	I	R	E	M	A
																		1567
1576	TGC	ACC	ACA	GGA	GTC	CTC	CAG	ACC	CTC	ACC	CTC	TTT	AAG	GGC	TTT	TTT	GAG	CTG
	C	T	T	G	V	L	Q	T	L	T	L	F	K	G	F	F	E	L
																		1621
1630	TTC	CCT	TCT	CTA	AGC	CAT	AAT	CTC	TTA	GTG	GAT	TGA	GCC	CTC	TTG	GAA	AGA	CTT
	F	P	S	L	S	H	N	L	L	V	D							
																		1675
1684	CTC	TGC	CAT	CCC	TTT	GCA	CCT	GAG	AGG	GGA	AGT	TCT	CAG	CTG	AGC	TGA	AGC	TGG
																		1729
1738	ATT	ATT	AAA	GTG	GAT	TGT	CAC	TCA	GAC	TCT	CCG	TGC	TAC	GCT	TAT	TTG	GAG	ACT
																		1783
1792	AGA	GGA	GTG	GGA	GTT	GAG	CCT	GGC	TTG	AAC	CTT	TGG	AAC	CAG	AAA	AGT	TGG	GGA
																		1837
1846	GCA	GGT	GGA	GGA	GGC	CAC	ACT	CCT	GGG	AGC	TGA	TGG	TTT	TAA	ATC	TGG	TTT	TAA
																		1891
1900	ATC	TCA	AAA	AAA	AAA	AAA	A	3'										
																		1909

FIGURE 4E

1 - - - - -
 1 KPACSSVRLTTREKPHCVCDPMTCPAKLGI2827886
 1 - - - - -
 31 LDQVCGTDNQTYTSSCYLFA TKCKLEGT KKGI2827886
 1 - - - - -
 61 GHQLQLITWSQLIYPACTDFEVTQFLRM RGI2827886
 1 - - - - -
 91 DWLKNISCSMNLTLNTLDISTRSRETKSRGI2827886
 1 - - - - -
 121 KSTWMKKRLLAGDHPIDLLREHRA TS RFGGI2827886
 1 - - - - -
 151 QVRQLLTCRGGAGCGARSGAQGGNGAEGI2827886
 1 - - - - -
 181 RLGRPGQASGAEASVSRRRRTCAFD TLEAFLGI2827886

FIGURE 5A

1	-	M D G E D I P D F S S L K E E T A Y W K E L S L K Y K Q S	031381
211	I	M D G E D I P D F S S L K E E T A Y W K E L S L K Y K Q S	GI 2827886
30	F	Q E A R D E L V E F Q E G S R E L E A E L E A Q L V Q A E	031381
241	S	R K A R D E L V E F Q E G S R E L E A E L E A Q L V Q A E	GI 2827886
60	Q	R N R D L Q A D N Q R L K Y E V E A L K E K L E H Q Y A Q	031381
271	Q	R N R D L Q A D N Q R L K Y E V E A L K E K L E H Q Y A Q	GI 2827886
90	S	Y K Q V S V L E D D L S Q T R A I K E Q L H K Y V R E L E	031381
301	S	Y K Q V S V L E D D L S Q T R A I K E Q L H S T C R E L E	GI 2827886
120	Q	A D D L E R A K R A T I V S L E D F E Q R L N Q A I E R	031381
331	Q	A D D L E R A K R A T I V S L E T L T - K L N Q A I E R	GI 2827886
150	N	A F L E S E L D E K E S L L V S V Q R L K D E A R D L R Q	031381
360	N	A F L E S E L D E K E S L L V S V Q R L K D E A R D L R Q	GI 2827886
180	E	L A V R E R Q Q E V T R K S A P S S P T L D C E K M D S A	031381
390	E	L A V R E R Q Q E V T R K S A P S S P T L D C E K M D S A	GI 2827886

FIGURE 5B

210	VQASLSLPATPVGKGTENTFPSPKAI	031381
420	VQASLSLPATPVGKGTENSFPSPKAI	GI 2827886
240	GTSP LTPSARI S A L N I V G D L L R K V G A L E S K	031381
450	GTSP LTPSARI S A L N I V G D L L R K V G A L E S K	GI 2827886
270	L A A C R N F A K D Q A S R K S Y I S G N V N C G V L N G N	031381
480	L A A C R N F A K D Q A S R K S Y I S G N V N C G V M N S N	GI 2827886
300	G T K F S R S G H T S F F D K G A V N G F D P A P P P G L	031381
510	G T K F S R S G H T S F F D K G A V N G F D P A P P P G L	GI 2827886
330	G S S R P S S A P G M L P L S V R V P S L Q V G A P A L L Q	031381
540	G S S R P L S A P G M C R S V C E C P A - - S G A P A L L Q	GI 2827886
360	Q P R T P T P H P S V P G P S P V P L R L P P H G W Q R A G	031381
568	Q P R T P T P H P S V P G P A L C P P S A S P H G W Q R A G	GI 2827886

FIGURE 5C

390	C	M	Q	W	R	L	L	G	P	A	Q	P	R	N	S	A	R	Y	Q	Y	W	L	F	S	L	L	A	V	V	P	031381
598	C	M	Q	W	R	C	F	G	P	A	Q	P	Q	D	S	A	R	Y	Q	Y	W	L	F	S	L	L	A	V	V	P	GI 2827886
420	L	V	S	H	D	C	T	F	V	G	R	K	V	I	H	T	C	I	T	W	S	L	D	A	E	V	P	I	H	H	031381
628	L	V	S	H	D	C	T	F	V	G	H	E	V	I	H	T	C	I	T	W	S	L	D	A	E	V	P	I	C	H	GI 2827886
450	T	C	P	I	A	P	T	L	L	Y																					
658	P	C	L	I	A	P	A	L	L	Y																					

FIGURE 5D

1	MWK	Q	I	G	A	Y	C	G	V	T	D	V	R	V	E	R	K	D	P	N	Q	V	E	L	W	G	1319265			
1	M	-	-	-	-	A	Q	L	C	G	L	-	-	-	-	-	R	R	S	R	A	F	L	A	L	G	GI 2065529			
31	L	K	E	G	T	Y	L	F	Q	L	T	V	T	S	S	D	H	P	E	D	T	A	N	V	T	V	L	1319265		
19	S	-	-	-	-	L	L	S	G	V	L	A	A	D	R	-	-	-	-	-	-	-	-	-	-	-	GI 2065529			
61	T	K	Q	T	E	D	Y	C	L	A	S	N	K	V	G	R	R	C	R	G	S	F	P	R	W	Y	D	1319265		
31	E	R	S	I	H	D	F	C	L	V	S	K	V	G	R	-	C	R	A	S	M	P	R	W	Y	N	V	GI 2065529		
91	E	Q	I	C	K	S	F	V	Y	G	G	C	L	G	N	K	N	Y	L	R	E	E	E	C	I	L	A	1319265		
60	D	G	S	C	Q	L	F	V	Y	G	G	C	D	G	N	S	N	N	Y	L	T	K	E	E	C	L	K	C	GI 2065529	
121	G	V	-	-	-	Q	G	P	L	R	G	S	S	G	A	Q	A	T	F	P	Q	G	P	S	M	E	R	H	1319265	
90	T	V	T	E	N	A	T	G	D	L	A	T	S	R	N	A	A	D	S	-	-	S	V	P	S	A	P	R	GI 2065529	
148	P	V	C	S	G	T	C	Q	P	T	Q	F	R	C	S	N	G	C	C	I	D	S	F	L	E	C	D	T	1319265	
118	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GI 2065529			
178	N	C	P	D	A	S	D	E	A	A	C	E	K	Y	T	S	G	F	D	E	L	Q	R	I	H	F	P	S	D	1319265
118	-	-	-	-	-	D	S	E	D	H	S	S	D	M	F	-	-	N	Y	E	-	-	-	-	-	-	-	-	GI 2065529	

FIGURE 6A

208	G	H	C	V	D	L	P	D	T	G	L	C	K	E	S	I	P	R	W	Y	Y	N	P	F	S	E	H	C	A	R	1319265	
132	-	Y	C	T	A	N	A	V	T	G	P	C	R	A	S	F	P	R	W	Y	F	D	V	E	R	N	S	C	N	N	GI 2065529	
238	F	T	Y	G	G	C	Y	G	N	K	N	N	F	E	E	Q	C	L	E	S	C	R	G	I	S	K	K	D	1319265			
161	F	I	Y	G	G	C	R	G	N	K	N	S	Y	R	S	E	A	C	M	L	R	C	-	-	-	-	-	-	-	GI 2065529		
268	V	F	G	L	R	R	E	I	P	I	P	S	T	G	S	V	E	M	A	V	A	V	F	L	V	I	C	I	V	V	1319265	
184	-	F	R	Q	Q	E	N	P	P	L	P	L	G	S	K	V	V	L	A	G	L	F	V	M	V	L	I	L	F	GI 2065529		
298	V	V	A	I	L	G	Y	C	F	F	K	N	Q	R	K	D	F	H	G	H	H	H	P	P	P	T	P	A	S	1319265		
213	L	G	A	S	M	V	Y	-	L	I	R	V	A	R	R	N	-	-	-	-	-	-	-	-	-	-	Q	E	R	A	L	GI 2065529
328	S	T	V	-	S	T	E	D	T	E	H	L	V	Y	N	H	T	T	R	P	L	1319265										
233	R	T	V	W	S	S	G	D	D	K	E	Q	L	V	-	-	K	N	T	Y	V	L	GI 2065529									

FIGURE 6B

1	MSDEEARQSGGSSQAGAVTVSDVQELMRRK	2057812
1	MSDEEARQSGGSSQAGVTVSDVQELMRRK	GI 2055256
31	EEIEAQIKANYDVLESQKIGMNEP LV DCE	2057812
31	EEIEAQIKANYDVLESQKIGMNEP LV DCE	GI 2055256
61	GYPRSDVDLYQVVRTARHNIICLQNDHKA VM	2057812
61	GYPRSDVDLYQVVRTARHNIICLQNDHKA VM	GI 2055256
91	KQVEEALHQLHARDKKEKQARDMAEAHKEAM	2057812
91	KQVEEALHQLHARDKKEKQARDMAEAHKEAM	GI 2055256
121	SRKLGQSESQGPPRAFAKVNNSISPGSPASI	2057812
121	SRKLGQSESQGPPRAFAKVNNSISPGSPASI	GI 2055256

FIGURE 7A

151	A	G	N	P	G	V	G	H	S	S	P	C	P	G	D	T	G	L	Q	V	D	D	E	I	V	E	F	G	S	V	2057812
151	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	Q	V	D	D	E	I	V	E	F	G	S	V	GI 2055256
181	N	T	Q	N	F	Q	S	L	H	N	I	G	S	V	V	Q	H	S	E	G	K	P	L	N	V	T	V	I	R	R	2057812
166	N	T	Q	N	F	Q	S	L	H	N	I	G	S	V	V	Q	H	S	E	G	-	A	L	A	P	T	I	L	L	S	GI 2055256
211	G	G	K	H	Q	L	R	L	V	P	T	R	W	A	G	K	G	L	L	G	C	N	I	I	P	L	Q	R	2057812		
195	V	S	M	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	T	P	G	T	S	S	R	S	P	GI 2055256		

FIGURE 7B

1 MNG - - KARKAEVQTAAKELLLKFFVNRSPSPF 2058485
1 MAAALKPSAPEIRKAAQEFINYLNKAVTFF GI 529706

29 HAVAEERNRLLQAGFSELKETEKWN IKPES 2058485
31 HATQEVKDRLLQAGFTLELPESGHWDIQPTIS GI 529706

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121 GGIWRTWFFDRDL SVA GLVIVK - - NGEK LQH GI 529706

149 QLVHVERPILRIPHLLAIHLQRNINENFGPN 2058485
149 KLIDVKKPVLFIPNLAIHLETD - RTTFKPN GI 529706

FIGURE 8A

179	T	E	M	H	L	V	P	I	L	A	T	A	I	Q	E	E	L	E	K	G	-	T	P	E	P	G	P	L	N	A	2058485
178	T	E	T	E	L	R	P	I	L	E	T	F	A	A	G	I	N	A	P	Q	K	P	E	S	T	G	F	A	D	GI 529706	
208	-	-	-	V	D	E	R	H	H	S	V	L	M	S	L	L	C	A	H	L	G	L	S	P	K	D	I	V	E	M	2058485
208	P	R	N	I	T	N	N	H	H	P	Q	F	L	G	L	I	A	K	E	A	G	C	P	E	D	I	V	D	L	GI 529706	
235	E	L	C	L	A	D	T	Q	P	A	V	L	G	G	A	Y	D	E	F	I	F	A	P	R	L	D	N	L	H	S	2058485
238	D	L	Y	L	Y	D	T	N	K	A	A	I	V	G	M	E	D	E	F	I	S	G	A	R	L	D	N	Q	V	G	GI 529706
265	C	F	C	A	L	Q	A	L	I	D	S	C	A	G	P	G	S	L	A	T	E	P	H	V	R	M	V	T	L	Y	2058485
268	T	Y	T	A	I	S	G	L	L	E	S	L	T	G	-	E	S	F	K	N	D	P	Q	I	R	I	A	A	C	F	GI 529706
295	D	N	E	E	V	G	S	E	S	A	Q	G	A	Q	S	L	L	T	E	L	V	L	R	R	I	S	A	S	C	Q	2058485
297	D	N	E	E	V	G	S	D	S	A	M	G	A	S	S	S	F	T	E	F	V	L	R	R	L	S	A	G	-	G	GI 529706
325	H	P	T	A	F	E	E	A	I	P	K	S	F	M	I	S	A	D	M	A	H	A	V	H	P	N	Y	L	D	K	2058485
326	S	T	T	A	F	E	E	A	I	G	K	S	M	L	I	S	A	D	Q	A	H	A	T	H	P	N	Y	S	A	K	GI 529706

FIGURE 8B

355	H E E N H R P L F H K G P V I K V N S K Q R Y A S N A V S E	2058485
356	H E E N H R P A F H G G V V K V N V N Q R Y A T T S T H	GI 529706
385	A L I R E V A N K V K V P L Q D L M V R N D T P C G T T I G	2058485
386	A A L K Q V A F E A Q V P L Q V V V V R N D S P C G S T V G	GI 529706
415	P I L A S R L G L R V L D L G S P Q L A M H S I R E M A C T	2058485
416	P I L A T K L G L Q T V D V G C P Q L A M H S I R E F A D T	GI 529706
445	T G V L Q T L T L F K G F F E L F P S L S H N L L V D	2058485
446	S S I Y Q A T T L Y S T F Y E R L S T V L S N M - - Q	GI 529706

FIGURE 8C

As a below named inventor, I hereby declare that:

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

the specification of which:

/ / is attached hereto.

/X/ was filed on May 1, 1998 as application Serial No. 09/071,709 and if this box contains an X
/ /, was amended on _____.

_____/ was filed as Patent Cooperation Treaty international application No. _____ on _____, 19____, if this box contains an X /____/, was amended on under Patent Cooperation Treaty Article 19 on _____ 19____, and if this box contains an X /____/, was amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 or §365(a)-(b) of any foreign application(s) for patent or inventor's certificate indicated below and of any Patent Cooperation Treaty international application(s) designating at least one country other than the United States indicated below and have also identified below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application for said subject matter the priority of which is claimed:

Country	Number	Filing Date	Priority Claimed
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)

I hereby appoint the following:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that

"Express Mail" mailing label number EL 579 911 818 US. I hereby certify that this document and referenced attachments are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, addressed to: Commissioner for Patents, Box Patent Application, Washington, D.C. 20231 on November 14, 2000.

By: Nancy Ramos

Printed: Nancy Ramos

JC-945 U.S. PTO
09/713669
11/15/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hillman et al.

Title: HUMAN PROTEASE ASSOCIATED PROTEINS

Serial No.: To Be Assigned

Filing Date: Herewith

Examiner: To Be Assigned

Group Art Unit: To Be Assigned

Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE UNDER 37 C.F.R. §3.73(b), REVOCATION OF POWER OF ATTORNEY AND APPOINTMENT OF NEW ATTORNEYS

Sir:

The undersigned has reviewed all the documents in the chain of title of the above-identified patent application and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

Incyte Genomics, Inc., formerly known as Incyte Pharmaceuticals, Inc., having a principal place of business located at 3160 Porter Drive, Palo Alto, California 94304, certifies that it is the assignee and owner of the entire right, title and interest in, to, and under the invention described and claimed in the above-identified application by virtue of an Assignment recorded at Reel 9402, Frame 0915, hereby revokes all previous powers of attorney and appoints the following patent attorneys/agents:

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The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

INCYTE GENOMICS, INC.

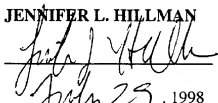
Date: November 14, 2000

By: Lee Bendekgey
Lee Bendekgey
VP, General Counsel/Corporate Secretary


such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sole Inventor or

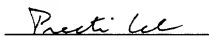
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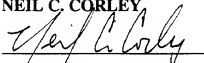
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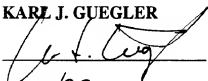
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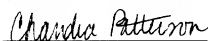
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Tang, Y. Tom
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Patterson, Chandra

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Arg Val Pro Ser	335	Leu Gln Val Gly Ala	340	Pro Ala Leu Leu Gln	345
Pro Arg Thr Pro	350	Thr Pro His Pro Ser	355	Val Pro Gly Pro Ser	360
Val Pro Leu Arg	365	Leu Pro Pro His Gly	370	Trp Gln Arg Ala Gly	375
Met Gln Trp Arg	380	Leu Leu Gly Pro Ala	385	Gln Pro Arg Asn Ser	390
Arg Tyr Gln Tyr	395	Trp Leu Phe Ser Leu	400	Leu Ala Val Val Pro	405
Val Ser His Asp	410	Cys Thr Phe Val Gly	415	Arg Lys Val Ile His	420
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 Thr Lys Gln Thr Glu Asp Tyr Cys Leu Ala Ser Asn Lys Val Gly
 65 70 75
 Arg Arg Cys Arg Gly Ser Phe Pro Arg Trp Tyr Tyr Asp Pro Thr
 80 85 90
 Glu Gln Ile Cys Lys Ser Phe Val Tyr Gly Gly Cys Leu Gly Asn
 95 100 105
 Lys Asn Asn Tyr Leu Arg Glu Glu Glu Cys Ile Leu Ala Cys Arg
 110 115 120
 Gly Val Gln Gly Gly Pro Leu Arg Gly Ser Ser Gly Ala Gln Ala
 125 130 135
 Thr Phe Pro Gln Gly Pro Ser Met Glu Arg Arg His Pro Val Cys
 140 145 150
 Ser Gly Thr Cys Gln Pro Thr Gln Phe Arg Cys Ser Asn Gly Cys
 155 160 165
 Cys Ile Asp Ser Phe Leu Glu Cys Asp Asp Thr Pro Asn Cys Pro
 170 175 180
 Asp Ala Ser Asp Glu Ala Ala Cys Glu Lys Tyr Thr Ser Gly Phe

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Asp Glu Leu Gln Arg	Ile His Phe Pro Ser	Asp Lys Gly His Cys			
	200		205		210
Val Asp Leu Pro Asp	Thr Gly Leu Cys Lys	Glu Ser Ile Pro Arg			
	215		220		225
Trp Tyr Tyr Asn Pro	Phe Ser Glu His Cys	Ala Arg Phe Thr Tyr			
	230		235		240
Gly Gly Cys Tyr Gly	Asn Lys Asn Asn Phe	Glu Glu Glu Gln Gln			
	245		250		255
Cys Leu Glu Ser Cys	Arg Gly Ile Ser Lys	Lys Asp Val Phe Gly			
	260		265		270
Leu Arg Arg Glu Ile	Pro Ile Pro Ser Thr	Gly Ser Val Glu Met			
	275		280		285
Ala Val Ala Val Phe	Leu Val Ile Cys Ile	Val Val Val Val Ala			
	290		295		300
Ile Leu Gly Tyr Cys	Phe Phe Lys Asn Gln	Arg Lys Asp Phe His			
	305		310		315
Gly His His His His	Pro Pro Pro Thr Pro	Ala Ser Ser Thr Val			
	320		325		330
Ser Thr Thr Glu Asp	Thr Glu His Leu Val	Tyr Asn His Thr Thr			
	335		340		345
Arg Pro Leu					

<210> 3
 <211> 238
 <212> PRT
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 2057812

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 Gly Ala Val Thr Val Ser Asp Val Gln Glu Leu Met Arg Arg Lys
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 Glu Glu Ile Glu Ala Gln Ile Lys Ala Asn Tyr Asp Val Leu Glu
 35 40 45
 Ser Gln Lys Gly Ile Gly Met Asn Glu Pro Leu Val Asp Cys Glu
 50 55 60
 Gly Tyr Pro Arg Ser Asp Val Asp Leu Tyr Gln Val Arg Thr Ala
 65 70 75
 Arg His Asn Ile Ile Cys Leu Gln Asn Asp His Lys Ala Val Met
 80 85 90
 Lys Gln Val Glu Glu Ala Leu His Gln Leu His Ala Arg Asp Lys
 95 100 105
 Glu Lys Gln Ala Arg Asp Met Ala Glu Ala His Lys Glu Ala Met
 110 115 120
 Ser Arg Lys Leu Gly Gln Ser Glu Ser Gln Gly Pro Pro Arg Ala
 125 130 135
 Phe Ala Lys Val Asn Ser Ile Ser Pro Gly Ser Pro Ala Ser Ile
 140 145 150
 Ala Gly Asn Pro Gly Val Gly His Ser Ser Pro Cys Pro Gly Asp
 155 160 165
 Thr Gly Leu Gln Val Asp Asp Glu Ile Val Glu Phe Gly Ser Val
 170 175 180
 Asn Thr Gln Asn Phe Gln Ser Leu His Asn Ile Gly Ser Val Val
 185 190 195
 Gln His Ser Glu Gly Lys Pro Leu Asn Val Thr Val Ile Arg Arg
 200 205 210
 Gly Gly Lys His Gln Leu Arg Leu Val Pro Thr Arg Trp Ala Gly
 215 220 225
 Lys Gly Leu Leu Gly Cys Asn Ile Ile Pro Leu Gln Arg

<210> 4
 <211> 471
 <212> PRT
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 2058485

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 Glu Leu Leu Lys Phe Val Asn Arg Ser Pro Ser Pro Phe His Ala
 20 25 30
 Val Ala Glu Cys Arg Asn Arg Leu Leu Gln Ala Gly Phe Ser Glu
 35 40 45
 Leu Lys Glu Thr Glu Lys Trp Asn Ile Lys Pro Glu Ser Lys Tyr
 50 55 60
 Phe Met Thr Arg Asn Ser Ser Thr Ile Ile Ala Phe Ala Val Gly
 65 70 75
 Gly Gln Tyr Val Pro Gly Asn Gly Phe Ser Leu Ile Gly Ala His
 80 85 90
 Thr Asp Ser Pro Cys Leu Arg Val Lys Arg Arg Ser Arg Arg Ser
 95 100 105
 Gln Val Gly Phe Gln Gln Val Gly Val Glu Thr Tyr Gly Gly Gly
 110 115 120
 Ile Trp Ser Thr Trp Phe Asp Arg Asp Leu Thr Leu Ala Gly Arg
 125 130 135
 Val Ile Val Lys Cys Pro Thr Ser Gly Arg Leu Glu Gln Gln Leu
 140 145 150
 Val His Val Glu Arg Pro Ile Leu Arg Ile Pro His Leu Ala Ile
 155 160 165
 His Leu Gln Arg Asn Ile Asn Glu Asn Phe Gly Pro Asn Thr Glu
 170 175 180
 Met His Leu Val Pro Ile Leu Ala Thr Ala Ile Gln Glu Glu Leu
 185 190 195
 Glu Lys Gly Thr Pro Glu Pro Gly Pro Leu Asn Ala Val Asp Glu
 200 205 210
 Arg His His Ser Val Leu Met Ser Leu Leu Cys Ala His Leu Gly
 215 220 225
 Leu Ser Pro Lys Asp Ile Val Glu Met Glu Leu Cys Leu Ala Asp
 230 235 240
 Thr Gln Pro Ala Val Leu Gly Gly Ala Tyr Asp Glu Phe Ile Phe
 245 250 255
 Ala Pro Arg Leu Asp Asn Leu His Ser Cys Phe Cys Ala Leu Gln
 260 265 270
 Ala Leu Ile Asp Ser Cys Ala Gly Pro Gly Ser Leu Ala Thr Glu
 275 280 285
 Pro His Val Arg Met Val Thr Leu Tyr Asp Asn Glu Glu Val Gly
 290 295 300
 Ser Glu Ser Ala Gln Gly Ala Gln Ser Leu Leu Thr Glu Leu Val
 305 310 315
 Leu Arg Arg Ile Ser Ala Ser Cys Gln His Pro Thr Ala Phe Glu
 320 325 330
 Glu Ala Ile Pro Lys Ser Phe Met Ile Ser Ala Asp Met Ala His
 335 340 345
 Ala Val His Pro Asn Tyr Leu Asp Lys His Glu Glu Asn His Arg
 350 355 360
 Pro Leu Phe His Lys Gly Pro Val Ile Lys Val Asn Ser Lys Gln
 365 370 375
 Arg Tyr Ala Ser Asn Ala Val Ser Glu Ala Leu Ile Arg Glu Val
 380 385 390
 Ala Asn Lys Val Lys Val Pro Leu Gln Asp Leu Met Val Arg Asn

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          395          400          405
Asp Thr Pro Cys Gly Thr Thr Ile Gly Pro Ile Leu Ala Ser Arg
          410          415          420
Leu Gly Leu Arg Val Leu Asp Leu Gly Ser Pro Gln Leu Ala Met
          425          430          435
His Ser Ile Arg Glu Met Ala Cys Thr Thr Gly Val Leu Gln Thr
          440          445          450
Leu Thr Leu Phe Lys Gly Phe Phe Glu Leu Phe Pro Ser Leu Ser
          455          460          465
His Asn Leu Leu Val Asp
          470

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<210> 5
<211> 2327
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: 031381

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<400> 5
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cttttgcacac attggaggct tctttgatca tggatgggta agatatacca gatttttcaa 180
gtttaaagcg gaaactgctt tattggaagg aactttccct gaagtataag caaagcttcc 240
aggaagaatc gtagtagcta gttgaattcc aggaaggaag cagagaatata gaagcagagt 300
tgaggcgaca attagtacag gctgaacaaa gaaatagaga cttgcaggct gataacacaa 360
gactgaataa tgaagtggag gctataaagg agaagctaga gcatcaatat gcacagagct 420
ataagcaggt ctacaggtta gaagatgatt taagtcagac tcgggccatt aaggagcagt 480
tgcataaata tgtgagagag ctggagcagg ccaacgacga cctggagcga gccaaaagg 540
caacaatagt ttcactggaa gactttgaac aaaggctaaa ccaggccatt gaacgaagt 600
cattttttaga aagtgaactt gatgaaaagg aatcttttgt ggtctctgta cacaggttaa 660
aggatgaagc aagagattta aggcaagaac tagcagttcg ggaagacaaa caggaagtaa 720
ctagaagaac ggctcctagc tctccaactc tagactgtga aaagatggac tcgcgcgtcc 780
aagcatcact ttctttgccg gctaccctcg ttggcaaggg aacggagaaac acttttccct 840
caccgaagcg tataccaaat ggttttggtt ccagtcacat aactccctct cgtatgatat 900
cagcactaaa catcgtgggg gatctcttac ggaaagttag ggctttagaa tccaaattag 960
cagcttcgag gaattttgca aaggaccaag catcacgaaa atctatatat tcagggaagt 1020
ttaactgtgg ggtgctgaat ggcaatggca caaagtcttc tgcagcaggg catacatctt 1080
tcttcgacaa aggggcagta aacggctttg acccgcctcc tcctctcctt ggtctggggt 1140
cttcgcgtcc cctgtcagcg ccgggtatgc tgcctctcag tgcgagatg tgcagcaggt 1200
aggtgtgggg tctgtccctc tcccaacaac ccaggacacc cagcgctcac ccctcggtgc 1260
ctgggcccag cccgctgccc ctccgctcgc ctccgacgg ctggcagagg cagcgctgca 1320
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gtccttcagt tcaacgacat ctttgagagt tttttgtttt tcttccagc ccgctgtctc 1860
ttgtgttagg aagggtgagt ggctggttcc aggggtggcc ggtgcagact ccggggtgga 1920
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tacatttgtt ttaattgtaca taggtttgta aacattattg cctgagatat ttgtatataa 2100
cttgggcttt gttagctttta tttattcaga acgcatacgg catgtttaat actctgatgg 2160
tgtctctctc tgggcagctg tataggatca tcatgtggtt acaaaaataa cttccctcaa 2220
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<210> 6
<211> 1870
<212> DNA

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<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 1319265

<400> 6

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ctgggtgctga aggatgtgga aaacacagat tggcgctctac tgcgggggtga caacggatgt 180
cagggtatagag aggaagaacc caaacagggt ggaactgtgg ggaactcaagg aaggaccta 240
cctgttccagc ctgacagtga ctagtctaga ccaccagag gacacggcca acgtcacagt 300
cactgtgctg tccaccaagg agacagaaga ctactgcctc gcatccaaca aggtgggtcg 360
cgcgtgcggg ggctcttccc cgcgctggta ctatgacccc acggagcaga tctgcaagag 420
ttctgtttat ggagggtgct tgggcaacaa gaacaactac cttcgggaag aagagtgcac 480
tctagctctg cggggtgtgc aaggtggggc tttagagagg agctctgggg ctcaaggcgac 540
tttccccagc ggccccctcca tggaaaggcg ccatccagtg tgcctgggca cctgtcagcc 600
acccagcttc cgtctgcagca atggctgctg catcgacagt ttcctggagt gtgacgacac 660
ccccaaactg cccagacctt ccgacgaggc tgcctgtgaa aaatacacga gtgctttgga 720
cagagctcag cgcacacctt tcccgcgctg gtactacaac ccttcaagg agcagcagtg 780
agagctctgc aaggagagca tcccgcgctg gtactacaac ccttcaagg agcagcagtg 840
cgcgttttacc taagggtggt gttatggcaa caagaacaac tttaggagg agcagcagtg 900
cctcgagttc tgttcgggca tctccaaaga ggaatgtgtt gccctgaggc ggaatattcc 960
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acacacacac caaccacac caccacctgc cagctccact gtctccacta ccgagacac 1140
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gagtggtggg gtgtcagacc ctggaggccc caacctgtc cctccgagct cctcttccat 1560
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ctggcttaga ccagcagaca gagccaggag aggcctcagc gcattccgca gcccccacc 1800
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aaaaaaaaa

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<210> 7

<211> 1138

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 2057812

<400> 7

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gcgggtcttc ttgagctcgc gcccggggtt cagcatgtcc gacgaggaag cgagcgagag 120
cggagctctc tgcgagggcg gcgcgctgac tgtcagcagc gtccaggagc tgcagcgcg 180
caagaggag atagaagcgc agatcaaggc caactatgac gtgctggaaa gccaaaaag 240
ctctgggatg aacgagccgc tggttgact ttgagggctac gcccggtcag acgtggacct 300
gtaccaagtc cgcaccgccca ggcaacacat catatgcctg cagaatgatc acaaggcagt 360
gatgaagcag gtgagaggag cctgcacac ctgtcacgct cgcgaacaag agaagcagcg 420
ccgggacatg gctgaggccc acaaaaggcg catgagccgc aaactgggtc agagtggag 480
ccagggccct caccggcctc tgcaccaagt gaacagcatc agccccgctc cccagcagc 540
catcgcgggt aatccagggg ttggccactc aagtccatgc ccaggggaca cgggtctgca 600
agtgtgatgat gagattttgg agttcggctc tgtgaacacc ggaacttcc agtcactgca 660
taacattggc agtgtgtgtg agcacagtga ggggaagccc ctgaattgta cagtgatccg 720
cagggggggc aaacaccagc ttgacttgt tccaacacgc tgggacggaa aagactgct 780
gggctgcaac attattcttc tgcaaaagat attgtccctg gggacagata acaggaaagc 840
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	50						55			60
Gly His Gln Leu	Gln	Leu	Ile	Thr	Trp	Ser	Leu	Gln	Ile	Tyr
	65					70				75
Ala Cys Thr Asp	Phe	Glu	Val	Thr	Gln	Phe	Pro	Leu	Arg	Met
	80					85				90
Asp Trp Leu Lys	Asn	Ile	Ser	Cys	Ser	Cys	Met	Asn	Leu	Thr
	95					100				105
Asn Thr Leu Asp	Ile	Ser	Thr	Arg	Ser	Arg	Glu	Thr	Lys	Ser
	110					115				120
Lys Ser Thr Trp	Met	Lys	Lys	Arg	Leu	Leu	Ala	Gly	Asp	His
	125					130				135
Ile Asp Leu Leu	Leu	Arg	Glu	His	Arg	Ala	Thr	Ser	Arg	Phe
	140					145				150
Gln Val Arg Gln	Leu	Leu	Thr	Cys	Arg	Gly	Gly	Ala	Gly	Cys
	155					160				165
Gly Ala Arg Ser	Gly	Ala	Gln	Gly	Cys	Gly	Gly	Asn	Gly	Ala
	170					175				180
Arg Leu Gly Arg	Pro	Gly	Gln	Ala	Ser	Gly	Ala	Glu	Ala	Ser
	185					190				195
Ser Arg Arg Arg	Thr	Cys	Ala	Phe	Asp	Thr	Leu	Glu	Ala	Phe
	200					205				210
Ile Met Asp Gly	Glu	Asp	Ile	Pro	Asp	Phe	Ser	Ser	Leu	Lys
	215					220				225
Glu Thr Ala Tyr	Trp	Lys	Glu	Leu	Ser	Leu	Lys	Tyr	Lys	Gln
	230					235				240
Ser Arg Lys Ala	Arg	Asp	Glu	Leu	Val	Glu	Phe	Gln	Glu	Gly
	245					250				255
Arg Glu Leu Glu	Ala	Glu	Leu	Glu	Ala	Gln	Leu	Val	Gln	Ala
	260					265				270
Gln Arg Asn Arg	Asp	Leu	Gln	Ala	Asp	Asn	Gln	Arg	Leu	Lys
	275					280				285
Glu Val Glu Ala	Leu	Lys	Glu	Lys	Leu	Glu	His	Gln	Tyr	Ala
	290					295				300
Ser Tyr Lys Gln	Val	Ser	Val	Leu	Glu	Asp	Asp	Leu	Ser	Gln
	305					310				315
Arg Ala Ile Lys	Glu	Gln	Leu	His	Ser	Thr	Cys	Arg	Glu	Leu
	320					325				330
Gln Ala Asn Asp	Asp	Leu	Glu	Arg	Ala	Lys	Arg	Ala	Thr	Ile
	335					340				345
Ser Leu Glu Thr	Leu	Thr	Lys	Leu	Asn	Gln	Ala	Ile	Glu	Arg
	350					355				360
Ala Phe Leu Glu	Ser	Glu	Leu	Asp	Glu	Lys	Glu	Ser	Leu	Leu
	365					370				375
Ser Val Gln Arg	Leu	Lys	Asp	Glu	Ala	Arg	Asp	Leu	Arg	Gln
	380					385				390
Leu Ala Val Arg	Glu	Arg	Gln	Gln	Glu	Val	Thr	Arg	Lys	Ser
	395					400				405
Pro Ser Ser Pro	Thr	Leu	Asp	Cys	Glu	Lys	Met	Asp	Ser	Ala
	410					415				420
Gln Ala Ser Leu	Ser	Leu	Pro	Ala	Thr	Pro	Val	Gly	Lys	Gly
	425					430				435
Glu Asn Ser Phe	Pro	Ser	Pro	Lys	Ala	Ile	Pro	Asn	Gly	Phe
	440					445				450
Thr Ser Pro Leu	Thr	Pro	Ser	Ala	Arg	Ile	Ser	Ala	Leu	Asn
	455					460				465
Val Gly Asp Leu	Leu	Arg	Lys	Val	Gly	Ala	Leu	Glu	Ser	Lys
	470					475				480
Ala Ala Cys Arg	Asn	Phe	Ala	Lys	Asp	Gln	Ala	Ser	Arg	Lys
	485					490				495
Tyr Ile Ser Gly	Asn	Val	Asn	Cys	Gly	Val	Met	Asn	Ser	Asn
	500					505				510
Thr Lys Phe Ser	Arg	Ser	Gly	His	Thr	Ser	Phe	Phe	Asp	Lys
	515					520				525
Ala Val Asn Gly	Phe	Asp	Pro	Ala	Pro	Pro	Pro	Pro	Gly	Leu
	530					535				540

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Ser	Ser	Arg	Pro	Leu	Ser	Ala	Pro	Gly	Met	Cys	Arg	Ser	Val	Cys	
				545					550					555	
Glu	Cys	Pro	Ala	Ser	Gly	Ala	Pro	Ala	Leu	Leu	Gln	Gln	Pro	Arg	
				560					565					570	
Thr	Pro	Thr	Pro	His	Pro	Ser	Val	Pro	Gly	Pro	Ala	Leu	Cys	Pro	
				575					580					585	
Pro	Ser	Ala	Ser	Pro	His	Gly	Trp	Gln	Arg	Ala	Gly	Cys	Met	Gln	
				590					595					600	
Trp	Arg	Cys	Phe	Gly	Pro	Ala	Gln	Pro	Gln	Asp	Ser	Ala	Arg	Tyr	
				605					610					615	
Gln	Tyr	Trp	Leu	Phe	Ser	Leu	Leu	Ala	Val	Val	Pro	Leu	Val	Ser	
				620					625					630	
His	Asp	Cys	Thr	Phe	Val	Gly	His	Glu	Val	Ile	His	Thr	Cys	Ile	
				635					640					645	
Thr	Trp	Ser	Leu	Asp	Ala	Glu	Val	Pro	Ile	Cys	His	Pro	Cys	Leu	
				650					655					660	
Ile	Ala	Pro	Ala	Leu	Leu	Tyr									
				665											

<210> 10

<211> 252

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: g2065529

<400> 10

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Leu	Leu	Gly	Ser	Leu	Leu	Leu	Ser	Gly	Val	Leu	Ala	Ala	Asp	Arg	
				20					25					30	
Glu	Arg	Ser	Ile	His	Asp	Phe	Cys	Leu	Val	Ser	Lys	Val	Val	Gly	
				35					40					45	
Arg	Cys	Arg	Ala	Ser	Met	Pro	Arg	Trp	Trp	Tyr	Asn	Val	Thr	Asp	
				50					55					60	
Gly	Ser	Cys	Gln	Leu	Phe	Val	Tyr	Gly	Gly	Cys	Asp	Gly	Asn	Ser	
				65					70					75	
Asn	Asn	Tyr	Leu	Thr	Lys	Glu	Glu	Cys	Leu	Lys	Lys	Cys	Ala	Thr	
				80					85					90	
Val	Thr	Glu	Asn	Ala	Thr	Gly	Asp	Leu	Ala	Thr	Ser	Arg	Asn	Ala	
				95					100					105	
Ala	Asp	Ser	Ser	Val	Pro	Ser	Ala	Pro	Arg	Arg	Gln	Asp	Ser	Glu	
				110					115					120	
Asp	His	Ser	Ser	Asp	Met	Phe	Asn	Tyr	Glu	Glu	Tyr	Cys	Thr	Ala	
				125					130					135	
Asn	Ala	Val	Thr	Gly	Pro	Cys	Arg	Ala	Ser	Phe	Pro	Arg	Trp	Tyr	
				140					145					150	
Phe	Asp	Val	Glu	Arg	Asn	Ser	Cys	Asn	Asn	Phe	Ile	Tyr	Gly	Gly	
				155					160					165	
Cys	Arg	Gly	Asn	Lys	Asn	Ser	Tyr	Arg	Ser	Glu	Glu	Ala	Cys	Met	
				170					175					180	
Leu	Arg	Cys	Phe	Arg	Gln	Gln	Glu	Asn	Pro	Pro	Leu	Pro	Leu	Gly	
				185					190					195	
Ser	Lys	Val	Val	Val	Leu	Ala	Gly	Leu	Phe	Val	Met	Val	Leu	Ile	
				200					205					210	
Leu	Phe	Leu	Gly	Ala	Ser	Met	Val	Tyr	Leu	Ile	Arg	Val	Ala	Arg	
				215					220					225	
Arg	Asn	Gln	Glu	Arg	Ala	Leu	Arg	Thr	Val	Trp	Ser	Ser	Gly	Asp	
				230					235					240	
Asp	Lys	Glu	Gln	Leu	Val	Lys	Asn	Thr	Tyr	Val	Leu				
				245					250						

<210> 11

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<211> 209

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: g2055256

<400> 11

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 20      25
Glu Glu Ile Glu Ala Gln Ile Lys Ala Asn Tyr Asp Val Leu Glu
 35      40
Ser Gln Lys Gly Ile Gly Met Asn Glu Pro Leu Val Asp Cys Glu
 50      55
Gly Tyr Pro Arg Ser Asp Val Asp Leu Tyr Gln Val Arg Thr Ala
 65      70
Arg His Asn Ile Ile Cys Leu Gln Asn Asp His Lys Ala Val Met
 80      85
Lys Gln Val Glu Glu Ala Leu His Gln Leu His Ala Arg Asp Lys
 95      100
Glu Lys Gln Ala Arg Asp Met Ala Glu Ala His Lys Glu Ala Met
110      115
Ser Arg Lys Leu Gly Gln Ser Glu Ser Gln Gly Pro Pro Arg Ala
125      130
Phe Ala Lys Val Asn Ser Ile Ser Pro Gly Ser Pro Ala Ser Ile
140      145
Ala Gly Leu Gln Val Asp Asp Glu Ile Val Glu Phe Gly Ser Val
155      160
Asn Thr Gln Asn Phe Gln Ser Leu His Asn Ile Gly Ser Val Val
170      175
Gln His Ser Glu Gly Ala Leu Ala Pro Thr Ile Leu Leu Ser Val
185      190
Ser Met Asn Leu Thr Thr Pro Gly Thr Ser Ser Arg Ser Pro
200      205
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<210> 12

<211> 470

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: g529706

<400> 12

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 20      25
His Ala Thr Gln Glu Val Lys Asp Arg Leu Leu Gln Ala Gly Phe
 35      40
Thr Glu Leu Pro Glu Ser Gly His Trp Asp Ile Gln Pro Thr Ser
 50      55
Lys Tyr Phe Val Thr Lys Asn Arg Ser Ala Ile Leu Ala Phe Ala
 65      70
Val Gly Gly Ser Tyr Lys Pro Gly Ser Gly Phe Ser Ile Val Val
 80      85
Gly His Thr Asp Ser Pro Cys Leu Arg Val Lys Pro Ile Ser His
 95      100
Gln Lys Ser Asp Lys Phe Leu Gln Val Gly Val Ser Thr Tyr Gly
110      115
Gly Gly Ile Trp Arg Thr Trp Phe Asp Arg Asp Leu Ser Val Ala
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	125				130				135
Gly Leu Val Ile	Val Lys Asn Gly Glu	Lys	Leu Gln His Lys	Leu					
	140				145				150
Ile Asp Val Lys	Lys Pro Val Leu Phe	Ile	Pro Asn Leu Ala	Ile					
	155				160				165
His Leu Glu Thr	Asp Arg Thr Thr Phe	Lys	Pro Asn Thr Glu Thr						
	170				175				180
Glu Leu Arg Pro	Ile Leu Glu Thr Phe	Ala	Ala Ala Gly Ile Asn						
	185				190				195
Ala Pro Gln Lys	Pro Glu Ser Thr Gly	Phe	Ala Asp Pro Arg Asn						
	200				205				210
Ile Thr Asn Asn	His His Pro Gln Phe	Leu	Gly Leu Ile Ala Lys						
	215				220				225
Glu Ala Gly Cys	Gln Pro Glu Asp Ile	Val	Asp Leu Asp Leu Tyr						
	230				235				240
Leu Tyr Asp Thr	Asn Lys Ala Ala Ile	Val	Gly Met Glu Asp Glu						
	245				250				255
Phe Ile Ser Gly	Ala Arg Leu Asp Asn	Gln	Val Gly Thr Tyr Thr						
	260				265				270
Ala Ile Ser Gly	Leu Leu Glu Ser Leu	Thr	Gly Glu Ser Phe Lys						
	275				280				285
Asn Asp Pro Gln	Ile Arg Ile Ala Ala	Cys	Phe Asp Asn Glu Glu						
	290				295				300
Val Gly Ser Asp	Ser Ala Met Gly Ala	Ser	Ser Ser Phe Thr Glu						
	305				310				315
Phe Val Leu Arg	Arg Leu Ser Ala Gly	Gly	Ser Thr Thr Ala Phe						
	320				325				330
Glu Glu Ala Ile	Gly Lys Ser Met Leu	Ile	Ser Ala Asp Gln Ala						
	335				340				345
His Ala Thr His	Pro Asn Tyr Ser Ala	Lys	His Glu Glu Asn His						
	350				355				360
Arg Pro Ala Phe	His Gly Gly Val Val	Lys	Val Asn Val Asn						
	365				370				375
Gln Arg Tyr Ala	Thr Thr Ser Thr Thr	His	Ala Ala Leu Lys Gln						
	380				385				390
Val Ala Phe Glu	Ala Gln Val Pro Leu	Gln	Val Val Val Val Arg						
	395				400				405
Asn Asp Ser Pro	Cys Gly Ser Thr Val	Gly	Pro Ile Leu Ala Thr						
	410				415				420
Lys Leu Gly Leu	Gln Thr Val Asp Val	Gly	Cys Pro Gln Leu Ala						
	425				430				435
Met His Ser Ile	Arg Glu Phe Ala Asp	Thr	Ser Ser Ile Tyr Gln						
	440				445				450
Ala Thr Thr Leu	Tyr Ser Thr Phe Tyr	Glu	Arg Leu Ser Thr Val						
	455				460				465
Leu Ser Asn Met	Gln								
	470								